DEVELOPMENT OF READY-TO-COOK CHICKEN CHIPS USING SPENT HEN MEAT INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

A Thesis
Submitted to the
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In partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

IN

POULTRY SCIENCE

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KHANAPARA, GUWAHATI-781022
July, 2022


Dedicated
To My
Beloved Parents

Academic Regulation Form No. PG-22
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ASSAM AGRICULTURAL UNIVERSITY

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All kinds of help received by her have been duly acknowledged.

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Communication of Decision of the Institutional Animal Ethics Committee (IAEC)

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was carried out to develop ready-to-cook chicken chips utilizing spent hen meat incorporated with fenugreek seeds and/or leaves powder. For this study twenty numbers of healthy spent hens were used following standard protocols for slaughtering and processing.

Fenugreek (*Trigonella foenum graecum*) seeds and its fresh leaves were purchased from local market of Guwahati city and processed to powdered form and stored for further use. The fenugreek leaves and the seeds were analyzed for proximate parameters. The fenugreek leaves contained 85.64 ± 0.72 % moisture, 4.62 ± 0.14% protein, 0.94 ± 0.01% ether extract, 1.69 ± 0.13% crude fibre and 10.73 ± 0.12% total ash. While the fenugreek seeds contained 10.26 ± 0.15% moisture, 26.86 ± 0.10% crude protein, 10.72 ± 0.15% ether extract, 47.52 ± 0.39% crude fibre and 3.82 ± 0.07% total ash.

The qualitative phytochemical studies of fenugreek seeds and leaves revealed presence of steroids, phenols, tannins, flavanoids, alkaloids and saponins.

The antioxidant activity against DPPH radical, total phenolic content and ferric reducing activity of the fenugreek seeds and leaves were studied using ethanolic extract. The mean per cent values of inhibition of DPPH radical by ethanolic extract were observed to be 51.40 ± 2.27 and 64.39 ± 1.73% for fenugreek leaves and fenugreek seeds, respectively. The total phenolic content in ethanolic extract of both fenugreek leaves and fenugreek seeds were recorded as 5.16 ± 0.06 and 15.13 ± 0.02 mg GAE/g, respectively. The mean (±SE) ferric reducing activity by ethanolic extract of both fenugreek leaves and fenugreek seeds were found to be 0.35 ± 0.03 and 0.65 ± 0.04, respectively and thus exhibit remarkable antioxidant activity.

The antibacterial activities of both extracts (fenugreek leaves and seeds) exhibited positive reaction against *Staphylococcus aureus* and *Klebsiella* spp. at different concentrations showing zones of inhibition ranging from 10 to 19 mm. The extracts of fenugreek seeds exhibited anti-bacterial effect against *E. coli* but no effect could be found with fenugreek leaves. Moreover, no antibacterial activity could be observed against *Salmonella* spp. by fenugreek leaves as well as fenugreek seeds.

The research trials were continued in two Phases, i.e., I and II. Under Phase I chicken chips was prepared as per standard formulation incorporating fenugreek seeds and/or leaves @ 0.25, 0.50 or 1.00 % level. The products were stored in sealed LDPE bags at ambient temperature (37 ± 2^{o}C) for a period of 30 days. The samples were evaluated for the physicochemical, proximate and sensory parameters at a regular interval of 10 days starting from 0th day, till 30th day.

The moisture level in all the treatment groups for fenugreek leaves as well as seeds including that of Control progressively increased as storage period extended till 30th day. The protein percentage in the treatment groups with increase in fenugreek seeds level (0.25, 0.50 and 1.00%) showed to have increased as compared to the Control and the values ranged from 22.33 ±0.22 to 22.85±0.09%. The analysis revealed no significant (P>0.05) differences among the various treatment groups incorporated with fenugreek seeds powder. The data analysis revealed no significant (P>0.05) differences in ether

extract among the various treatment groups incorporated with fenugreek leaves powder and control group and with increasing storage period. Significant changes ($P<0.05$) in total ash content could be noted in the Treatment groups with fenugreek seeds with increase in the level of fenugreek seed powder (0.5 and 1.0%).

Significant increase in pH could be seen on 30^{th} day of storage in all the treatment groups including that of Control. The impact of storage could not be noticed in the products made of, either leaves or seeds in terms of tyrosine value. The water activity remained unchanged till the 20^{th} day of storage, however increased significantly ($P<0.05$) on 30^{th} day whereas no change observed among the treatment groups for both fenugreek leaves and seeds addition. The cooking yield of 90.97 ± 0.76% to 95.00 ± 1.77% range was recorded in the chicken chips incorporated with fenugreek leaves and fenugreek seeds powder.

The freshly prepared chicken chips with addition of fenugreek leaves and seeds on day 1 exhibited 'good' colour, texture, crispiness scores under hedonic scale. The sensory evaluation of the chicken chips product treated with fenugreek seeds and leaves powder showed low for flavor, after-taste scores and overall acceptability in the Treatment II (FL with 0.50%), III (FL with 1.00%) and Treatment V (FS with 0.50%) & VI (FS with 1.00%) groups throughout the storage period of 30 days.

Under Phase I trial, based on the statistical analysis obtained, two best groups FL with 0.25% and FS with 0.25% along with combination of both (FL+FS with 0.25 each) were selected for further studies. All the physicochemical values for the treatment groups were found to be under desirable ranges. Significant increase in the moisture level was found on 30^{th} day of storage as compared to the 0^{th}, 10^{th} and 20^{th} day however, no changes were observed among different treatment groups.

The crude protein values ranged from 22.36 ± 0.02 to 23.03 ± 0.06% among all treatment groups. Significantly ($P<0.05$) high crude protein was recorded in the Treatment A (FS with 0.25%) and Treatment C (FL, FS 0.25% each) when compared with Control. The ether extract and total ash content in chicken chips revealed non-significant ($P>0.05$) changes when compared with the Control group.

Storage days showed significant ($P<0.05$) effect on pH of the products and treatment with combination of fenugreek leaves and seeds significantly showed lower ($P<0.05$) pH on 30^{th} day when compared with 0^{th} to 20^{th} day of storage. There was no significant difference between the treatment groups and Control group throughout the storage period and the values remained far below permissible limit for all the products. The analysis of variance showed significant difference ($P<0.05$) in water activity values on 30^{th} day of storage compared to the a_w on the 0^{th}, 10^{th} and 20^{th} day of storage. There was no significant ($P>0.05$) change in cooking yield of the treatment groups with increase in storage period and among the different treatment groups. The TBA values decreased significantly ($P<0.05$) on the 10^{th} day of storage and remained static thereafter up to 30 days of storage. The cholesterol content of ready-to-cook chicken chips using spent hen with addition of fenugreek seeds and fenugreek leaves are found to be as 30.55 ± 0 .14, 30.45 ±0.21, 30.39 ± 0.16 and 31.44 ± 0.14% for Control, T-A, T-B and T-C, respectively with no significant ($P>0.05$) changes among the groups. The colour profile for the chicken chips showed significant differences only in L* values while no changes observed in a* and b* values. A significant increase in mean DPPH activity was noted in

all the treatment groups incorporated with fenugreek leaves and seeds powder revealing its potential antioxidant capacity.

The total plate count analyzed for the products were within the limits and were free from Coliform, Salmonella, Staphyloccocal bacteria and yeast and mould which ensures the microbial safety of the product.

No significant difference was noticed for colour, texture, crispiness characteristics among the Control and treatment groups but could retain ‘good’ to ‘very good’ scores for the product. The chicken chips under all treatment groups scored very less scores in terms of flavour, after-taste and overall acceptability with increase in levels of fenugreek leaves and seeds powder. The chicken chips prepared with the incorporation of spent hen and fenugreek leaves or seeds have revealed good antioxidant profile without any noticeable changes in any other physico-chemical parameters and microbiological profile. Fenugreek leaves at 0.25% level can be effectively used in chicken chip preparation using spent hen meat with ‘good’ acceptability having cost of production of ` 7.45 per 30g of the product. It could be concluded that a level of 0.25% fenugreek leaves powder can effectively be incorporated in production of ready-to-eat chicken chips as functional food having added health benefits.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
ANOVA	Analysis of Variance
g	Gram
Kg	Kilogram
Kcal	Kilo Calorie
ml	Mililitre
mg	Miligram
µg	Microgram
Lit.	Litre
M	Molar
Mm	Milimolar
Cfu	Colony forming unit
IU	International Unit
%	Per cent
₹	Rupees
OD	Optical density
^{0}C	Degree Celsius
SE	Standard Error
FL	Fenugreek leaves
FS	Fenugreek seeds
DPPH	2,2-diphenyl-1-picryhydrazyl
TPC	Total Plate Count
YMC	Yeast and Mould Count
IC	Inhibition Concentration
n	number
-ve	negative
+ve	positive
ND	Not detected
LDPE	Low density polyethylene
RH	Relative humidity
TBA	Thioarbarbitric acid
MDA	Malondialdehyde

CHAPTER - I

Introduction

DEVELOPMENT OF READY-TO-COOK CHICKEN CHIPS USING SPENT HEN MEAT INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

CHAPTER-I

INTRODUCTION

Snack food has always remained a part of Indian consumption way of life. The demand for snack food market has increased in recent times. Snack foods are mostly food of choice for single person households, school going children and high mobility populations (Lusas and Rooney, 2001). The Indian snack market worth around 5.57 billion U.S. dollars in 2020 compared to 6.25 billion U.S. dollars in the previous year. The snack market in India mostly includes chips, proved a rising development and is projected to attain almost 13 billion US dollars by 2026 (Statista, 2022). Chips are a well known snack food and one of the most unique and universal fast food items. There are various chips available in the market either in ready-to-eat or ready-to-cook form such as potato chips, banana chips, corn chips, apple chips, cassava chips and tortilla chips. Munching on chips happened to be an essential part of the consumption practice of the majority of the people mostly the growing young population. Major factors driving the global demand of chips are growing urbanization, income increase, changing socio-economic pattern of life, habit of eating out, convenience in terms of time and effort, increasing working couples and rapidly changing lifestyles (Sharma *et al.*, 2018). Long working lifestyle for most of the members of family have forced to change from regular meals to fast foods, snacks and packaged foods. Countries worldwide have faced numerous consequences due to the Covid-19 outbreak both in positive and negative ways. Government restrictions, lockdown, shutting down of businesses and income losses are reasons behind disruption of economy. However, the demand for some snacks have grown-up at the time of Covid-19 pandemic when nationwide lockdown was imposed. Snacks such as chips, tortilla and others have grown in demand as people were more concerned about stocking up food edibles with long shelf life and ready to cook products (Food and Beverage, 2021). Chips are the convenient snack item easily available with long shelf life and contribute towards energy intake of the consumers. These chips are generally prepared from natural ingredients, good at taste but contained high calorie and low protein per cent. Therefore it is high need for alternative ingredients to be used to the original recipes and introducing new interesting products in terms of flavours, textures and producing healthy version of chips. Its beneficial properties can be enhanced when prepared with meat having high quality protein.

Indian layer industry has witnessed a remarkable expansion and thereby spent hen availability has also increased many folds in the recent past. According to Jacob *et al*., 2014, worldwide, billions of spent hens are produced every year. But management costs of these spent hens in India have created a need for alternatives. One such alternative is the utilization of these spent hen meat to produce cheaper and economically viable nutritious products. The laying hens at the end of their active life period are considered as byproducts of the egg industry. Presence of high collagen makes the spent hen meat tough, poor in functional properties and less juicy when tasted (Baily, 1984) Thereby it is not much preferred by the consumers unlike broilers and roasters. To minimize the disposal problems of spent hen, these are mostly utilized in pet food industry and preparation of value added meat products such as soup, snack, sausages, nuggets, meatballs, salami etc (De Souza *et al.,* 2011) being a good protein source. Moreover snacks made with meat are more nutritious than products made with vegetable proteins. New technologies need to be developed to boost the utilization of spent hen meat which can unleash new avenues for improving the profitability of the layer industry.

Chicken meat chips are ready- to-eat product prepared with addition of various types of vegetable extenders or binders. Incorporation of extenders having high starch and crude fiber in meat products is also beneficial for the digestibility and health benefits of consumers. Binder or extenders make an essential component of value added products. Utilization of such material for meat product depends upon the ability to absorb moisture in the dough making process and to retain its shape throughout the preparation process.

The incidence of lifestyle diseases such as obesity, cardiac problems, hypertension and diabetes have increased due to change in food habits, lifestyle and food content. Hence, a rise in the need of alternatives in the form of functional food products has aroused suitable for the modern consumers. In order to avoid obesity and all diseases related to the ongoing pandemic, it can help to establish healthy eating habits and assess the nutritional quality of products (De Vlieger *et al.,* 2017). The products prepared from

spent hen meat can be nutritionally enhanced with the inclusion of different ingredients like flour, starch and milk proteins and other natural food additives (Tarte *et al.*, 1989).

The oxidation process of spent hen meat occurs at a faster rate compared to broiler during processing and storage due to presence of high unsaturated fatty acids (Qureshi *et al.,* 2018). In order to lessen the oxidative rancidity, use of synthetic antioxidants has been restricted due to its negative heath effects on consumers. Hence it has become necessary to look for other natural solutions to lessen the deterioration and extending the shelf-life of meat products (Saito *et al*., 2003). Growing awareness among consumers about healthy diet has increased the interest on the use of natural plants in foods which are found to contain antioxidants compounds. Use of natural antioxidants in the form of herbs and spices can replace the chemical preservatives and their toxic effects. Use of such medicinal herbs makes the product functional promoting health benefits. Natural antioxidants found in the plants have gained a considerable momentum for their role in preventing the auto-oxidation in foods (Reddy *et al.* 2005). One such herb is the Fenugreek (*Trigonella Foenum-graecum L.)* is one such miraculous herb, seeds and leaves of which are used as powder or extract for medicinal purposes due to its antioxidant as well as antimicrobial properties. According to Petropoulos (2002), the species name "*foenum-graecum"* means "Greek-hay" which indicated that it was used as a forage crop in the past. Two commonly known species of Fenugreek viz., *T. foenum graecum* (common methi) and *T. corniculata* (kasuri methi) were found to have economic importance (Moradi kor and Moradi, 2013).

Fenugreek belongs to the plant family of Fabaceae and originally from Southeastern Europe and Western Asia. Commonly known as 'methi' in India, it is grown in many parts of the world (Altuntas *et al.*, 2005). It is mostly used as a spice in many culinary recipes and serves as a good anti-diabetic, lactation stimulant and hypocholesterolaemic (Mohamed *et al*., 2015).

The therapeutic properties of fenugreek can be extensively exploited for the development of functional food product. Various phytochemicals compounds aiding to its restorative properties such as alkaloids, steroids, flavonoids and amino acids like 4-hydroxyproline, isoluecine, histidine, arginine and lysine are found in fenugreek. The

seeds contain chemical constituents such as, coumarin, fenugreekine, nicotinic acid sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its therapeutic effect (Singh *et al.*, 2022). It also contained a soluble fiber called galactomannan that helps reducing blood cholesterol and sugar level (Nematollahi *et al.,* 2016). The organoleptic properties including texture of the product can be modified with the incorporation of fenugreek. Fenugreek has been examined as preservative in many foods items specifically in meat during its process to replace chemical preservatives, but there is barely any research done based on the antimicrobial and antioxidant activities in products having fenugreek leaves and seeds. Phytochemical study indicates that the fenugreek leaves are rich sources of phytochemicals which have a wide range of biological effects including antioxidant and antimicrobial properties.

Information on meat chips incorporated with functional compounds and natural additives are very scanty. So considering the above facts, the present study was planned to develop chicken chips prepared using spent hen meat and evaluate their quality attributes incorporated with suitable level of Fenugreek leaves and Fenugreek seed powder added with different non-meat ingredients in the standardized recipe with the following objectives:

1. To standardize the recipe for ready-to-cook chips using spent hen meat with different levels of fenugreek seeds and/or leaves powder.
2. To study the various physico-chemical and organoleptic characteristics of the formulated ready- to-cook chicken chips.
3. To study the effect due to addition of fenugreek leaves and/or seed on the antioxidant activity and microbial content of the chips.
4. To study the keeping quality of ready-to-cook chicken chips stored at room temperature.
5. To estimate the cost of production of developed ready-to-cook chicken chips.

CHAPTER - II

Review of Literature

PERFORMANCE OF LARGE WHITE YORKSHIRE ON FEEDING BANANA STEM AND MIXED (BANANA STEM AND TARO) SILAGE FERMENTED WITH PROBIOTICS

CHAPTER-II

REVIEW OF LITERATURE

2.1 PRESENT SCENARIO OF SPENT HEN

Chips are one of the most unique and preferred nutrient fast food items. Use of meat in preparation of chips have shown to include additional properties in terms of nutrients. Comminution of spent hen meat into a value added products like chicken meat chips have boost better utilization of tough, unpalatable, less juicy spent hen meat. (Nagamallika and Rao, 2010).

With incredible growth in layer industry, the spent hen availability has increased many folds in the recent past. Billions of spent hens are produced annually, worldwide (Jacob *et al.*, 2014). In India, spent hens are further processed into various products or snacks, such as nuggets, cutlet, jerky, patty, tikka and others (Rajeswar *et al*., 2018, Sabikun *et al.*, 2021). Several meat based snacks had been developed by Sharma and Sharma (2013). Spent hen meat powder incorporated papad (Gokulakrishnan, 2014) and bhujia (Talukder, 2015) were found self-stable with high sensory acceptance stored aerobically at ambient for 45 days.

Mishra *et al.* (2015) prepared chicken meat rings with utilization of spent hen meat. Usage of 10% of different extenders like rice flour, millet flour replacing lean meat without compromising the quality products.

Sorapukdee *et al.* (2016) carried out an experiment to develop dried jerky exploiting spent hen meat. Addition of humectants such as glycerol and sorbitol at the concentration of 0, 10 and 15% and roasting could enhance the quality of the jerky by improving the sensory scores of color, appearance, flavor and overall acceptability.

Reshi *et al.* (2017) undertook a trial to evaluate the effect of addition of ginger extract on the shelf life of spent hen meat. The parameters determined were physico-chemical, proximate analysis and sensory evaluation.

Indumathi *et al.* (2019) studied the utilization of less costly spent hen meat to produce economically viable nutritious value added chicken sausages.

Sapcota (2020) carried out trials to develop iron enriched spent hen meat products such as meatballs and nuggets from layer industry and for entrepreneurship development.

Snacks are like small meal available in the market in diverse forms, shapes and taste (Tettweiller, 1991). These are convenient foodstuffs and consumers relish these due to its variety of taste and calorific values (Weinstock, 1989). With rapid urbanization, change in lifestyles and peoples' mindset, consumption of snacks had increased day by day (Lusas and Roney, 2001). The volume of only snack food segment is expected to hike up to 17,086.6 m Kg by 2027 with a growth of 5.4% in 2023 (Statista, 2022). The Indian snack market exhibited robust growth during 2016-2021 expects the market to grow at 12% during 2022-2027 (Market Analysis Report, 2022).

According to APEDA (2006) survey report, there are about 1000 snack items and 300 types of savories snacks sold in India presently. The market for branded chips and wafers alone stands at Rs. 1100 crore presently.

2.2. DEVELOPMENT OF MEAT BASED CHIPS OR SNACKS

Berwal *et al*. (1996) developed urkey meat papads using raw and heat treated (50^{o}C/20 mins) meat by blending with rice flour (50:50). Traditional rice papads were taken as control. They observed that heat treated turkey meat blended papads had significantly higher sensory scores and better acceptibility.

Sharma and Nanda (2002) made efforts to develop chips utilizing spent hen meat. The four formulations namely were prepared containing chicken meat at the level of 95, 85, 80 and 75 per cent. The chicken chips developed proved to be stored under aerobic condition up to 12 weeks.

Singh *et al.* (2022) prepared chicken snacks with spent hen meat concluded that the sensory scores indicated an improving trend with increase in level of meat in the mix.

Das and Jayaraman (2003) developed a dehydrated chicken pulav from chicken meat and preserved under ambient and chill temperature in pouches of polypropylene film (PP) and paper-foil-polyethylene laminate (PFP). Results showed that the product could be preserved with a shelf-life of 8-12 and 14-18 months under ambient and chill temperature, respectively.

Popped cereal snacks using various combinations of spent hen meat and grains (potato starch, corn starch, and rice flour) were developed (Lee *et al.*, 2003). Electron microscopic and optical microscopic observations revealed the popping degree of snack with starch and spent hen meat increase with presence of meat.

Crawford and Crawford (2004) prepared chicken skin chips made of natural chicken skin composed with or without various flavoring spices, then baked or deep-fried. It was found that the crunchy chicken skin chips that are dried at temperatures up to 250^0 F can be packed in vacuum-sealed containers and preserve the product until consumption.

Mckee *et al*. (2007) evaluate the physical and sensory properties of chilli flavoured, puffed, extruded products made from defatted chopped beef, mechanically deboned chicken or chicken thigh meat and coated with cheese seasoning containing three levels of chilli and potato flour.

Rajamohamed *et al*. (2007) prepared dehydrated chicken meat chunks and assrssed its storage quality in two different packaging materials like Low density polyethylene (LDPE) and Metalised polypropylene pouches (MPP). Study revealed that the dehydrated product can be effectively stored in metallised polypropylene laminated pouches with extended shelf life at room temperature for period of 2 months.

Nagamallika and Rao (2010) developed chicken meat papads incorporated with vegetable binder and found that the addition of bengal gram and black gram flours was effective in chicken meat papad preparation.

Microwaved ready-to-eat snacks prepared from meat of different species such as chicken, chevon, mutton and pork were studied in a research trial (Meshram *et al*., 2012). Based on the mean values of physico-chemical parameters obtained from microwaved snacks prepared from mutton were found significantly better than others.

Biswas *et al.* (2015) aimed to study and standardize the meat level (80,70 and 60%) and type of meat suitable for development of meat finger chips using turkey and spent hen meat. It was concluded that meat finger chips containing 70% meat mince at 50:50 ratio of turkey and spent hen meat was most suitable based on different physico-chemical and sensory quality characteristics.

Mishra *et al.* (2015) took up a study to develop dehydrated chicken meat rings using spent hen meat and different extenders such as rice flour, barnyard millet flour and texturized soy granule powder at 5, 10 and 15% levels. It was concluded that product with 10 % rice flour had a yield of 41.89% and it had 10.28% fat and 37.82% protein in it.

Talukder *et al.* (2015) studied on the nutritional quality, storage stability and acceptability of traditional bhujia with the incorporation of spent hen meat powder. Their findings found that the bhujia was suitable to be aerobically stored at ambient condition for 45 days without deteriorating its quality and acceptability.

Singh *et al.* (2015a) development of ready-to-eat chicken meat caruncles using spent hen. Meat caruncles prepared using 65% spent hen meat and 0.5% baking powder showed all the sensory attributes in desirable range along with significantly higher cooking yield, moisture, hardness and crispiness.

Kasthuri *et al.* (2017) studied in a trial on the effect of adding 1% drumstick leaf powder, 1% jamun seed powder and 0.5% drumstick leaf powder + 0.5% jamun seed powder in chicken chips. It showed non-significant changes on physicochemical, sensory and microbiological quality of chicken chips and can be stored effectively for 30 days at room temperature without any deterioration in qualities.

Kasthuri *et al.* (2018) prepared chicken chips incorporated with different levels (2, 4 and 6%) of flaxseed powder (FSP) and (3, 6 and 9%) oats powder (OP). Three cooking methods were involved deep fat frying, microwave cooking and hot air oven cooking and were compared. Based on sensory quality FSP level of 4% and OP 6% were selected for the development of functional chicken chips under microwave cooking.

Nemade and Londhe (2018) undertook a study to standardize the suitable quality of cookies with incorporation of various levels of spent hen meat powder (0%, 50%, 60% and 70%) packed in LDPE bags in aerobic condition under ambient temperature of 37±1 ^{0} C. With increase in storage period up to 60 days, the physicochemical attributes such as pH, protein, ash, fat and sensory scores showed significant decrease while moisture content increased.

Lindasari *et al.* (2021) found out the effect of Moringa leaf flour on the characteristics and nutritional value of chicken sausage chips. The treatment group incorporated with Moringa leaf flour at the level of 0%, 1%, 2% and 3%. The results showed that with addition of Moringa leaf flour, on the chemical characteristics such as water content, ash content, protein, and fat have increased significantly ($P<0.05$).

2.3. PROXIMATE COMPOSITION OF FENUGREEK SEED AND FENUGREEK LEAVES

Hemavathy and Prabhakar (1989) estimated dry seeds of fenugreek to contained 7.5% total lipids, 84.1% neutral lipids, 5.4% glycolipids and 10.5% phospholipids.

Gopalan *et al.* (1992) observed the results for analysis of fenugreek leaves as moisture 86.73±0.66%, protein 3.68±0.36%, ash 1.69±0.19%, fat 0.83±0.02%, fibre 4.90±0.21% and energy 144±10 Cal.

The raw seeds of fenugreek contained higher amount of dietary fiber (46.50%) according to Hooda and Jood (2003) than fenugreek leaves extract.

Srinivasan (2006), in his experiment analyzed certain components of fenugreek leaves as follows.

Parameters	Percent
Moisture	86.0
Protein	4.4
Fat	1.0
Fibre	1.0

Trivedi *et al.* (2007) analyzed the chemical composition of fenugreek seed and found to have 20-25% proteins, 45-50% dietary fiber, 20-25% soluble fiber, 6-8% fatty acids and essential oils, and 2-5% steroidal saponins.

Shakuntala *et al.* (2011) reported that the protein percentage of germinated endosperm, sprouts and ungerminated endosperm of fenugreek seeds were 39.25%, 36.12% and 48.20%, respectively. However, insoluble and total dietary fibres were recorded to be 55.80% and 86.96% in germinated seed coat, whereas 31.90% and 77.10% respectively in ungerminted seed coat.

Al-Jasass and Al-Jasser (2012) conducted an experiment on chemical composition of fenugreek seeds and found the crude fiber content ranged from 6.36 to 23.6% while ash content ranged from 3.57 to 7.1%. Fenugreek seeds contained 23–26% protein, 6–7% fat and 58% carbohydrates of which about 25% was dietary fiber (US Department of Agriculture, 2012).

According to study conducted by Singh *et al.* (2013) revealed that ash content in fenugreek seeds ranged from 3.0 to 3.87%. The fiber content varied from 5.6 to 8.93%, of different fenugreek genotypes. In another study, protein content in seeds varied from 18.1 to 24.63% with an average mean performance of 20.78 %. (Singh *et al.*, 2013)

Mullaicharam *et al.* (2013) reviewed on the physicochemical composition of whole fenugreek seeds and defatted seeds and found as follows:

Proximate composition (%) of fenugreek seeds	Whole seeds	Defatted seeds
Moisture	9.0	9.0
Ash	3.0	3.5
Lipids	8.0	Negligible

Protein	26.0	28.3
Starch	6.0	6.5
Total Fiber	48.0	51.7
Gum	20.0	19.2
Neutral detergent fiber	28.0	32.5

Agrawal *et al.* (2015) conducted an experiment on physico-chemical properties of fenugreek seeds and revealed that raw seeds of fenugreek had 7 % fat, 23.30 % protein and 3 % ash content.

Buba *et al.* (2015) aimed at determining the physicochemical properties of fenugreek seed and found the moisture and ash contents values as 10.91±0.85% and 2.99±0.48%, respectively. The protein content showed 27.4±0.35%, while fat contents found to be 6.33±0.52%.

Bhatnagar and Azhar (2016) studied ash content of fenugreek leaves to be 10.30 ± 0.3 g/100g. Fenugreek leaves found to contain about 86.1% moisture, 4.4% protein, 0.9% fat, 1.5% minerals, 1.1% fiber, and 6% carbohydrates (Wani and Kumar, 2016).

Dilshad (2017) studied about the physicochemical properties of fenugreek seeds in four different geographic locations. The values of moisture and oil contents were recorded higher in fenugreek seed as 5.47 ± 0.66 and 7.04 ± 0.21 respectively.

Mahmood and Yahya (2017) revealed in a study about the moisture, crude fiber , total ash, total oil, crude protein, carbohydrate, nitrogen content and caloric value of fenugreek seed on a dry weight basis to be 6.833±0.531 %, 17.0±0.2%, 3.566±0.478%, 7.15±0.25%, 28.45±0.15%, 1340±0.029 mg/100g, 5544.9 Kcal/100g respectively.

Joshi *et al.* (2019) showed the results of the chemical analysis of the fenugreek leaves puree as moisture (86.90 per cent), crude fat (1.1 per cent), protein (2.84 per cent), carbohydrate (5.95 per cent) crude fiber (1.75 per cent) and ash (1.56 per cent).

Singh *et al.* (2020) found that 100 g of fenugreek seeds contained protein 6.3%, fat 9.5%, carbohydrates 42.3%, volatile oil 7%, vitamin A 1040 IU and calorific value of 370 Cal.

Alane *et al.* (2021) evaluated the chemical composition of fresh fenugreek leaves and dried fenugreek leaves. The fresh fenugreek leaves comprised 84.51 per cent moisture, 0.71 per cent fat, 8.23 per cent carbohydrate, 3.75 per cent protein, 1.22 per cent ash and 1.64 per cent crude fibre. While the dried fenugreek leaves contained 8.21 per cent moisture, 4.81 per cent fat, 70.9 per cent carbohydrate, 8.27 per cent protein, 2.83 per cent ash and 4.98 per cent crude fibre.

2.4. ANTIOXIDANT ANALYSIS OF FENUGREEK LEAVES AND SEEDS

Hwa *et al.* (2019) investigated on the antioxidant activity of fenugreek using DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging activity assay and total phenolic content test to determine the total amount of phenolic compound of the seed extract.

Akbari *et al.* (2019) studied the antioxidant activity against 2,2-diphenylpicrylhydrazyl (DPPH) of fenugreek seed oil indicated a strong antioxidant radical scavenging activity against DPPH assays with an IC50 of 172.6 ± 3.1 and suggested that the fenugreek seed oil could be used for pharmaceutical purposes.

Rahmani *et al.* (2018) worked on a phyto-chemical study to determine and compare the amount of phenolic compounds and antioxidant activity of four different cultivars of fenugreek seeds grown at the National Institute for Agricultural Research

(INRA) in Lamtar (Sidi Bel Abbes, North West Algeria). The total phenolic content in the extracts was determined using the Folin-Ciocalteu reagent and it ranged between 1,613 and 2,083 gallic acid equivalents mg/g of extract.

Yadav and Chowdhury (2017) undertook a study to examine the antioxidant activity of methanolic extract of seeds of *Trigonella foenum-graecum* seeds using DPPH free radical scavenging assay. The IC50 (The concentration of sample required to scavenge 50% of DPPH free radical) was calculated by plotting graph between % inhibition vs concentration. The Ascorbic acid was used as standard antioxidant in comparison to methanolic extract of *Trigonella foenum-graecum*. The IC50 value of extract and Ascorbic acid was found to be 3.24 µg/ml whereas IC50 value of methanolic extract was found to be 1.98 µg/ml. This suggests that methanolic extract of Fenugreek seeds had potent antioxidant activity.

Al-Maamari *et al.* (2016) evaluated the diversity of certain phytochemicals (total phenolic contents, flavonoids, saponins and tannins,) in seeds of twenty Omani fenugreek accessions. Significant ($P<0.05$) variability was observed in the mean values for total phenolic contents (TPC) in seeds of various fenugreek accessions.

Shinde *et al.* (2015) prepared Crude extract of fenugreek by soxhelt extraction method with different solvents(methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate).Extracts were used for measurement of total phenolic content (TPC) by Folin-Ciocalteu method, flavonoid content and antioxidant/radical scavenging activity(1,1-diphenyl-2-picryl-hydrazyl (DPPH))free radical scavenging activity. The result reveals that all fenugreek extracts could act as potent source of antioxidants.

Mashkor (2014) In a study,3 types of solvent extract of fenugreek seeds were used to examine the effects of extraction solvent on total phenolics content (TPC), DPPH and ferric reducing antioxidant power (FRAP) were found in 50% acetone extracts. The TPC for fenugreek seeds from 25.90-15.45 mg GAE/100g DW and antioxidant activity FRAP from 47.49-31.85 mg TE/100 g DW, DPPH were from 67.30%-43.61%). The largest amount of total phenol content which leads to more effective radical scavenging effect was shown by 50% acetone extract. Amount of phenolic compounds and

antioxidant activities increased in acetone extract. Acetone 50% and methanol 50% solvent showed the greatest capability in extracting antioxidants and inhibiting the free radicals produced. It was concluded that extraction solvent play important roles on the phenolics compounds and their antioxidant compounds and their antioxidant activity of fenugreek seeds extract.

Pathak *et al.* (2014) show the antioxidant activity of *Trigonella foenum-graecum.* Methanolic extract of fenugreek seeds were evaluated for total phenolics content, antioxidant activity using various assay systems such as (III) reduction, inhibition of hydroxyl radical mediated 2-deoxy-D- ribose degradation , metal ion (Fe+) chelation assay and DPPH radical scavenging assay. The results indicate that fenugreek is a very efficient antioxidant.

Ali and ElNou (2014) aimed to study antioxidant activity, total phenolic, flavonoid and tannin contents of methanolic and petroleum ether extracts of callus and seeds of fenugreek (*Traigonella foenum-graecum).* Free radical scavenging activity was evaluated by DPPH method where the highest scavenging activity was obtained from cotyledons derived callus (91.5±0.16%) followed by hypocotyls derived callus (85.46±0.29%) and methanolic extract of seeds (80.53±0.01%). Whereas petroleum ether extract of seeds demonstrated weak antioxidant activity. IC50 values of DPPH scavenging capacity of methanolic extracts were evaluated in descending order, plant seeds (1.1185mg/g)>hypocotyls derived callus (0.7159mg/g)>cotyledons derived callus (0.4914mg/g)> ascorbic acid (0.1874mg/g). Highest phenolic content were observed in callus of cotyledons (412.087 mg/l), compared to callus of hypocotyls (211.1937 mg/l) and methanolic extract of seeds (124.84 mg/l) calculated as mg/l gallic acid equivalent of phenols.

Total phenolics and flavonoids contents were obtained for methanolic and aqueous solvent extracts for each of the samples Antioxidant activity of the extracts was estimated using ABTS scavenging assay and FRAP assay (Pasricha and Gupta, 2014)

Sravanthi *et al.* (2013) study aims on antioxidant potential in *Trigonella foenum-graecum* L. (Ajmer methi variety). Leaves have been used for the determination and

quantification of antioxidant constituents and free radical scavenging activities for treatment and prevention of various disease like diabetes mellitus, atherosclerosis, cataract, rheumatism, cancer and other auto immune disease like ageing and antioxidants like phenols, flavonoids, flavonols, proanthocyanin, anthocyanin, total carotenoid, β-carotene, antioxidant enzyme systems like catalase, peroxidase, polyphenol oxidase, glutathione reductase activities and free radical scavenging assays like FRAP, ABTS, DPPH were evaluated. The result showed highest phenolic content 38.3±0.5mg/g dry wt. and FRAP free radical scavenging was 10±0.05 % recorded maximum than the other assays.

Priya *et al.* (2011) showed significant total antioxidant capacity of the fenugreek seeds with IC50 value of 192 µg/ml and hydroxyl radical with IC50 value of 587.5µg/ml and concluded that hydroalcoholic extract of *Trigonella foenum graecum* may have potential antioxidant effects against several oxidants.

Fenugreek seeds extracts were subjected for the measurement of total phenolic content (TPC) by Folin-Ciocalteu method as well as, reducing power and antioxidant/radical scavenging activity (DPPH). Results from different parameters were in agreement with each other and reveal that all extracts of the fenugreek exhibit antioxidant activity. Thereby results suggested that the fenugreek seed extract could act as potent source of antioxidants. (Bukhari *et al.*, 2008)

Kaviarasan *et al.* (2007) an extract of fenugreek seeds was isolated for antioxidant activity using various in vitro assay systems. The seed extract exhibited scavenging of hydroxyl radicals (OH) and inhibition of hydrogen peroxide induced lipid per oxidation. The extract at high conc. acted as scavenger of DPPH and ABTS. -radicals. The total phenolic content in the extract was determined spectrophotometrically according to the Folin-Ciocalteau procedure and expressed as mg gallic acid equivalents. The results indicate that the extract of fenugreek seeds contains antioxidants and protects cellular structures from oxidative damage.

Ghaskadbi and Devasagayam (2005) *Trigonella foenum-graecum* has antioxidant properties were studied in germinated fenugreek seeds which are considered to more

beneficial than dried seeds. Different fractions of the germinated seeds were used to determine their antioxidant potential at different levels. The assays employed were ferric reducing antioxidant power, radical scavenging by 1, 1-diphenyl-2-picrylhydrazyl, ferrylmyoglobin /2, 2–azobis-3-ethylbenzthiazoline-6-sulfonic acid, oxygen radical absorbance capacity and inhibition of lipid per oxidation in mitochondrial preparations. An aqueous fraction of fenugreek exhibited the highest antioxidant activity.

Randhir *et al.* (2004) the antioxidant activity estimated by DPPH assay indicate that fenugreek sprout extract can quench the superoxide free radical and scavenge the hydrogen peroxide generated in the reaction mixture.

2.5. PHYTOCHEMICAL COMPOSITION OF FENUGREEK SEEDS AND LEAVES

Fenugreek cultivation in India is mainly concentrated in Rajasthan and other surrounding regions with a total contribution of 83 % of the total fenugreek production in the country (Anonymuos, 2013). During 2017-18 fenugreek was cultivated in an area of 220 thousand hectares with production of 311 thousand MT (Anonymuos, 2018; Singh *et al.*, 2020).

Fenugreek seeds and leaves are rich in flavonoids and phenolic compounds having anti-inflammatory, anti-carcinogenic, antioxidant, antiviral and hypocholesteremic properties (Moradi and Moradi, 2013). Fenugreek seeds are a rich source of fiber (50–65 g/100 g) consisting mainly of non-starch polysaccharides (Montgomery, 2009).

Seeds consist of various saponins namely diosgenin, tigogenin, yamogenin and gitogenin (Taylor *et al.* 1997). The saponin content constituted 4 to 8% along with 1% alkaloid named Trigonelline responsible for the bitter taste of the seeds (Fatima *et al.* 2018).

Saponins and alkaloids are considered as anti-nutritional factors in seeds. However, defatted seeds are free from these compounds and may be consumed by people having problem with fat (Altuntas *et al.,* 2005). The fenugreek seeds contained considerable amounts of steroidal sapogenin with diosgenin which has great demand in pharmaceutical industry for production of steroidal drugs and sex hormones as well as in making oral contraceptives (Jayadev *et al.,* 2004).

Phytochemical analysis conducted by Khamees *et al.* (2022) showed the presence of many secondary metabolites including alkaloids, flavonoids, anthraquinones, tannins, and steroids, in the crude extracts of fenugreek seeds.

Bouhenni *et al*. (2021) conducted a research trial to determine the phytochemical profile of Algerian fenugreek seeds in order to characterize its phenolic compounds and to determine its antioxidant activities. The higher amounts of total phenolic compounds, flavonoids, condensed and hydrolysable tannins were showed by fenugreek seeds. Using HPLC method, the fenugreek seed extract showed to consist of seven molecules which support its good antioxidant properties.

Malik *et al*. (2020) and Shesharao *et al.* (2020) carried out a two independent qualitative analysis on the various bioactive components of alcoholic extracts of fenugreek which confirmed the presence of compounds such as steroids, flavanoids, saponin, tannin, alkaloids, anthracene, glycoside derivatives and coumarin.

Benziane *et al.* (2019) did a phytochemical study on both aqueous and ethanolic extracts of fenugreek in order to determine the total polyphenols by the method of Follin ciocaltchu. The total alkaloids and tannins confirmed their presence in the extracts and supported their antioxidant activity.

Khan *et al.* (2019) carried out a phytochemical evaluation to estimate the presence of carbohydrates, glycosides, flavonoids, tannins, phytosterols and phenolic compounds in different extracts (water, ethanol, chloroform and petroleum ether) of fenugreek seeds. Results proved the presence of these compounds as follows:

Test	Petroleum ether	Chloroform	Alcohol	Water
Alkaloids	-ve	-ve	+ve	-ve
Flavonoids	-ve	-ve	+ve	-ve
Steroids	+ve	+ve	-ve	-ve
Tannin	+ve	-ve	+ve	+ve
Saponin	-ve	-ve	+ve	+ve

Mahmood and Yahya (2017) used aqueous and alcoholic extract for analyzing the phytochemical, nutrient and active groups composition of fenugreek seeds powder. The extract appeared to contained several compounds such as alkaloids, flavonoids , steroids , carbohydrates, trepenes , tannins , saponins , glycosides , free amino acid , crude protein and phenolic compounds.

Saha (2017) validated the presence of various phytochemicals such as carbohydrates, proteins, glycosides, steroids, phenols, saponins, alkaloids and flavonoids as major active constituents of different extract of air, oven and microwave dried samples of fenugreek leaves and seeds by qualitative phytochemical tests.

In another research trial conducted by Sheikh *et al.* (2012) proved the presence of glycosides, phenol, flavonols, amino acid, alkaloides, steroids, tannin, polysaccharide, pectin and hemicelluloses, fats volatile oil in the ethanolic extract of fenugreek seeds. Whereas Dande and Patil (2012) on a phytochemical analysis observed that the steroidal saponins were in large quantity in fenugreek seed extract.

Sumayya *et al.* (2012) conducted a phytochemical analysis of leaves, stem and seeds of different extracts of fenugreek which revealed that it contained high content of sterols and alkaloids in all whereas the flavonoids content was in moderate level in leaves, seeds and absent in stem.

Nutrients	*Trigonella foenum-graecum*		
	Leaf	Stem	Seed
Phenols	+	-	++
Catechol	-	-	-
Steroids	++	++	++
Flavonoids	+	-	+
Alkaloids	++	++	++

Ahirwar and Ahirwar (2010) while working with petroleum extract, ethanolic extract and aqueous extract for phytochemical analysis revealed presence of steroids in fenugreek seeds only in petroleum extract and absent in aqueous and ethanolic extracts. Whereas Yadav *et al.* (2010) showed the presence of alkaloid, flavonoids, amino acid, tannins, protein, starch, mucilage and saponins in the methonolic and aqueous extracts of fenugreek.

Mowla *et al.* (2009) subjected crude ethanol extract of fenugreek seeds for phytochemical analysis and the study findings showed the presence of alkaloid, steroid and carbohydrate but absence flavonoids, glycoside and glucosides in the crude seed extract.

2.6. ANTIMICROBIAL ANALYSIS OF FENUGREEK LEAVES AND SEEDS

Hwa *et al.* (2019) carried out agar well diffusion method to estimate the antimicrobial activity of fenugreek by measuring the zone of inhibition on nutrient agar. The extract concentrations which used in this method were 10mg/ml, 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ ml and 100mg/ml. screening for antimicrobial activity, showed that all the concentrations of fenugreek have negative results in *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 8739), and *Pseudomonas aeruginosa* (ATCC 27853) except for *Bacillus subtilis* (ATCC 6633).

Tailor and Jain (2018) aimed to study the comparative antimicrobial analysis of fenugreek leaves, stems, seeds of mature and immature plant, also dried plant (Kasoori methi) against gram-negative and gram-positive bacteria using different solvents acetone and methanol using agar well diffusion method. The result showed that methanolic extract was more effective than acetonic extract and Kasoori methi showed largest zone of inhibition in bacterial culture of *Bacillus subtilis* (3.46 mm) in methanolic extract, while no significant zone observed in the brown seeds.

Al-Timimi (2019) worked on the utilization of fenugreek seed as an antibacterial agent and results showed that the highest activity of the extract of the seed was on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (22 mm and 17 mm diameter of inhibition zones, respectively).

The present investigation was performed to evaluate the antimicrobial potential of *Trigonella foenum-graecum* leaves extracts and to elucidate the presence of phytochemicals responsible for its biological activity.

Massih *et al.* (2018) aimed to determine the antimicrobial activity of three selected plants (*Rosmarinus officinalis*, *Origanum majorana*, and *Trigonella foenum-graecum*) against Extended Spectrum Beta Lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumonia* and to identify the specific plant fraction responsible for the antimicrobial activity. Ethyl acetate extracts of all selected plants exhibited relatively low Minimum Inhibitory Concentrations (MIC).

Sharma *et al.* (2017) studied the antimicrobial activity of Fenugreek leaves, seeds and stem extracts (Methanol, Acetone and aqueous extract) against *E. coli* and *Staphylococcus* determined by the well diffusion method. The maximum zone of inhibition was given by methanol i.e. 20 mm and 19 mm against *E. coli* and *Staphylococcus* respectively, followed by Acetone extract which give the equal zone of inhibition for both organism i.e. 16 mm while the aqueous extract shows nil zone of inhibition.

Kumar *et al.* (2013) aimed to screen the antibacterial activities of distilled water, methanol, acetone; ethanol extract of the spice. The *in vitro* antibacterial activity was performed by agar well diffusion method. Methanol, acetone, ethanol and distilled water extract of Fenugreek revealed an elevated antimicrobial activity against *Bacillus Subtilis* and *Candida parapsilosis* at lower concentration of the crude extract. The results obtained in the present study suggest that the methanol extract of fenugreek revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formation.

Phytochemical screening of the extracts revealed the presence of the major compounds known to have anti-bacterial activity such as tannins and flavonoids in the aqueous and methanolic extracts. The results indicate that fenugreek seeds crude extracts may have anti-bacterial potential against *E. coli* depending on the solvent used for the extraction. (Chalghoumi *et al.,* 2016).

Dharajiya *et al.* (2016) performed a trial to evaluate the antimicrobial potential of *fenugreek* leaves extracts and to study the presence of phytochemicals. The antibacterial activity of *fenugreek* leaves extracts was found maximum on *Serratia marcescens* with a zone of inhibition (ZOI) of 12.33Â±0.57 mm by aqueous extract followed by inhibition of *Bacillus cereus* (ZOI = 11.50Â±0.50 mm) by the methanol extract.

ElNour *et al.* (2015) aimed to investigate the antimicrobial activities and phytochemical screening of methanolic and petroleum ether extracts of seeds and callus derived from hypocotyls and cotyledons explants of fenugreek. Antimicrobial activities were tested against standard microorganisms, *Bacillus subtilus* (NCTC 8236 G+Ve), *Staphylococcus aureus* (ATCC 25923 G+v), *Escherichia coli* (ATCC 25922 G-V), *Pseudomonas aeruginosa* (ATTC 27853 G-V), *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596) using paper disc diffusion method. The petroleum ether extract of *T.foenum- graecum* seeds showed highest antimicrobial activity compared to methanolic extracts. Antibacterial activity of petroleum ether extract of *T. foenum-graecum* seeds were recorded (17±0.33mm) and (15±0.57mm) of inhibition zone against *Escherichia coli* and *Staphylococcus aureus* respectively by concentration 250 mg/ml.

Alwhibi and Soleman (2014) in a study, obtained fenugreek plants from two different cultivars, one from Saudi Arabia and another from Yemen, were screened for phytochemical active constituents and investigated for antimicrobial properties against a selection of grampositive and gram-negative pathogenic bacteria. Five different solvent seed extracts from each cultivar were tested. The results of the study demonstrated that the chloroform and methanolic extracts possessed significant antibacterial activity against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Salmonella typhi* ATCC14027 and *Klebsiella pneumonia* ATCC700603. The antimicrobial activity of the extracts was investigated using the agar well diffusion method. The results of the antimicrobial analysis identified *Shigella sonnei* as the most sensitive pathogen to the crude extracts of Fenugreek seeds obtained from Saudi Arabia and with the largest zones of inhibition.

Marzougui *et al.* (2012) have evaluated the antibacterial efficacy of crude aqueous and organic extracts of seeds, leaves and stems of *T. foenum-graecum* against a variety of bacterial species including the antibiotic resistant ones and found to be significantly effective.

Nandagopal *et al.* (2012) evaluated the phytochemical analysis for antibacterial activity of the seed extracts of fenugreek against pathogenic bacteria like Gram +ve *(Staphylococcus aureus)* and Gram -ve *(E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia)* bacteria by *in vitro* agar well diffusion method. The seed extracts showed more inhibitory action on *Klebsiella pneumonia* and *Pseudomonas aeruginosa* than *E. coli, Staphylococcus aureus* .

Abdalah (2011) studied the antibacterial activity of fenugreek seeds extract using agar diffusion against different bacterias such as *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeuroginosa, E. coli, Pseudomonas vulgaris, Klebsiella pneumonia.* The extract with concentration 1000, 500 and 250 mg/ml inhibited the growth of the bacteria *Streptococcus pyogenes.* The methanolic extract of fenugreek seeds with concentration of 1000, 500, 250 and 125 mg/ml that effectively inhibited the growth of *Staphylococcus aureus.*

Premnath *et al.* (2011) screened the antibacterial activity of various extracts of fenugreek leaves using 10 mg/ml concentration by disc diffusion method and the ethanolic extract was found to be most potent against *E coli, Proteus mirabilis, Klebsiella spp, Staphylococcua aureus, Pseudomonas aeroginosa, Enterobacter aerogens.*

Al-abdeen *et al.* (2010) tested the antibacterial activity of aqueous and some organic compounds extracts of stems, leaves, seeds and roots of fenugreek against 3 Gram –ve and 1 Gram +ve bacteria by well diffusion and colony count methods. The microorganisms used were *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and Klebsiella spp.* All extracts of the plant did not exhibit any inhibitory activity against any of the microorganisms tested by each well diffusion and colony account technique.

Khanra *et al.* (2010) in a study prepared ethanolic extract of fenugreek seeds and evaluated for antimicrobial activity against eight bacterial strains by determining zone of inhibition. The results revealed that the ethanolic extract is potent in inhibiting bacterial growth of both Gram +ve an Gram -ve bacteria.

Basu *et al.* (2009) compared the effectiveness of fenugreek against two common pathogenic bacteria. Fenugreek was found to strongly inhibit the growth of *Staphylococcus aureaus* and *Pseudomonus aeruginosa* in a petri dish.

Upadhyay *et al.* (2008) observed that the acetone extract of fenugreek showed promising inhibitory effect against *B. cereus, L. acidophilus* and *Pneumococcus.*

2.7. USE OF FENUGREEK IN MEAT AND MEAT PRODUCTS

Ahmad *et al.* (2019) analyzed the lipid oxidation and microbial spoilage of mutton and beef cattle meat on postmortem refrigerated storage due to various levels of crude fenugreek leaves (0, 0.5, 1.0 and 1.5%). Treatment with increasing level of fenugreek leaves had significantly lower content of malondialdehyde (MDA) at day 7 and 10 postmortem, respectively.

Qureshi *et al.* (2018) studied the usage of fenugreek seeds powder to boost the overall functional properties of spent hen meat patties incorporated at four different levels; 0.5%, 1%, 1.5% and 2% replacing lean meat in the formulation of the product. The study revealed that the use of fenugreek seed powder up to 1.5% can be very well be incorporated in meat products in order to improve its shelf life.

Zaki (2018) evaluated the effect of adding fenugreek seed powder at the rate of 5, 10 and or 15% in preparation of rabbit sausage on the quality attributes during frozen storage at 18°C±1 for 3 months period. The research trial showed improvement in the physicochemical properties, reduction in lipid oxidation and cooking loss with time. The product also proved to have significant sensory scores than control.

Hegazy (2011) evaluated the effect of application of as antioxidant and antimicrobial agent in production of beef burgers replacing soybean flour. The findings confirmed about the improvement in essential amino acids content, physicochemical qualities (pH, water holding capacity, cooking loss and thiobarbituric acid value) during frozen storage, as well as improvement of the microbiological quality. Also, beef burger samples containing fenugreek seed flour (3 and 6%), exhibited good sensory scores and better overall acceptability after frozen storage for 3 months.

McCarthy *et al.* (2001a) evaluated the antioxidant activities of aloe vera, fenugreek, ginseng, mustard, rosemary, sage and tea catechins in pork patties. These ingredients were more effective in reducing lipid oxidation in patties made from frozen (−20 °C) than fresh pork.

2.8. PROXIMATE ANALYSIS OF SPENT HEN CHIPS

2.8.1. Moisture

Lindasari *et al.* (2021) aimed to determine the effect of adding Moringa leaf flour at the rate of 0, 1, 2 & 3% on chicken sausage chips on the chemical attributes and nutritional value. The study concluded that with increase in level of Moringa leaf flour in the product, the water content of the chicken sausage chips have showed significant increase.

Dehydrated chicken meat rings were developed by Mishra and his co workers (2015) with 5 %, 10% and 15 % level of rice flour, barnyard millet flour and texturized soy granule respectively. The product found that the moisture content with 15% level of rice flour had significantly ($P < 0.05$) lower values than the control, and other two treatment products. However, no significant difference ($P > 0.05$) was observed among other products.

Singh *et al.* (2011) studied the physico-chemical characteristics of dried chicken snacks packaged aerobically and under vacuum in laminated pouches and stored for 3 months. The results obtained showed non- significant differences ($P>0.05$) in the contents of moisture, fat, protein and ash in both of the packaging systems.

Nagamallika and Rao (2010) prepared chicken meat papad with raw and partially cooked spent hen meat with addition of cooked mashed potato, bengal gram flour and black gram flour each at 15% level and evaluated for its quality. Papad with 15% bengal gram flour showed significantly lower moisture than those with mashed potato black gram flour, and control.

Sharma and Nanda (2002) made efforts to develop chips utilizing spent hen meat. The four formulations namely were prepared containing chicken meat at the level of 95, 85, 80 and 75 per cent. Moisture per cent in formulation with 95% meat content was found significantly higher ($P<0.05$) as compared to other formulations.

2.8.2. Protein

Lindasari *et al.* (2021) aimed to determine the effect of adding moringa leaf flour at the level of 0, 1, 2 & 3% on chicken sausage chips. The protein content of chicken sausage chips with the addition of moringa leaf flour for each treatment was significantly different ($P<0.05$).

Mishra *et al.*(2015) developed chicken meat ring using rice flour, bamyard millet flour and texturized soy granule and observed that protein per cent of dehydrated chicken meat rings prepared with optimum level of texturized soy granule was significantly

higher (P <0.05) than control as well as from products with optimum level of rice flour and bamyard millet flour

Nagamallika and Rao (2010) prepared chicken meat papad with raw and partially cooked spent hen meat with addition of cooked mashed potato, Bengal gram flour and black gram flour each at 15% level and evaluated for quality. Papad with 15% black gram flour showed significantly higher protein value than those with mashed potato, Bengal gram flour and control.

Sharma and Nanda (2002) developed chips utilizing meat from spent hen. The formulations were namely I, II, III and IV were made containing chicken meat at 95, 85, 80 and 75 per cent respectively. Crude protein contents in formulation I were significantly higher (P<0.05) as compared to other formulations.

Singh *et al.* (2011) prepared chicken snacks with spent hen meat concluded that the protein contents indicated an increasing trend with increase in level of meat in the mix.

Berwal *et al.* (1996) prepared papad using raw and heat treated (at 50^{o}C) turkey meat mixing with rice flour (50:50). Results of the study reported that there was significant increase in protein content of turkey raw meat and heat treated turkey meat papads as compared to control. The percent ash content of control papads was significantly higher, as compared to turkey raw meat and heat treated turkey meat papads.

2.8.3. Ether extract

Lindasari *et al.* (2021) aimed to determine the effect of adding Moringa leaf flour at 0, 1, 2 & 3% level on chicken sausage chips. The statistical analysis of ether extract averaged between 14.37-15.50%. The increase in fat content in chicken sausage chips for each treatment was significantly different. These results can be interpreted as adding moringa leaf flour to the chicken sausage chips showed increase in fat content.

Mishra *et al.* (2015) found the ether extract of dehydrated chicken meat rings prepared with optimum level of incorporation of rice flour (10%), bamyard millet flour (10%) and texturized soy granule powder (5%), were significantly lower ($P<0.05$) than control. Product with 5% level of texturized soy granule powder had significantly lower ($P<0.05$) value than control but had significantly higher ($P <0.05$) value of fat per cent than products with 10% level of rice flour and bamyard millet flour.

Nagamallika and Rao (2010) prepared chicken meat papad with raw and partially cooked spent hen meat with addition of cooked mashed potato, Bengal gram flour and black gram flour, each at 15% level and evaluated for quality. Papad with 15% mashed potato showed significantly lower ether extract than those with black gram flour, Bengal gram flour and control.

2.8.4. Total Ash

Lindasari *et al.* (2021) aimed to determine the effect of adding moringa leaf flour in four treatment groups (0, 1,2, and 3%) of chicken sausage chips. The results showed that the ash content for each treatment were significantly higher ($P<0.05$), with addition of higher level of moringa leaf flour to the chicken sausage.

Ash percentage of dehydrated chicken meat rings prepared with optimum level of incorporation of rice flour and bamyard millet flour were significantly lower ($P<0.05$) than control. Product with optimum level of texturized soy granule powder had non-significantly change ($P >0.05$) in the value than control but had significantly higher ($P <0.05$) value for ash percentage than the products with optimum level of rice flour and bamyard millet flour (Mishra *et al.,* 2015).

Berwal *et al.* (1996) prepared papad using raw and heat treated turkey meat blending with rice flour. The percent ash content of control papads was significantly higher, as compared to turkey raw meat and heat treated turkey meat papads.

2.9. PHYSICOCHEMICAL ANALYSIS OF DRIED MEAT PRODUCTS

2.9.1. pH

Physicochemical characteristics of meat products such as pH, water activity and composition are greatly affected by bacterial load of the products during storage (Bhardwaj *et al.*, 1995).

Kasthuri *et al.* (2017) formed four experimental groups of chicken chips incorporated with 1% drumstick leaf powder (T1), 1% jamun seed powder (T2) , 0.5% drumstick leaf powder + 0.5% jamun seed powder (T3) and Control group on physicochemical properties of chicken chips. Significant ($P<0.05$) differences in pH values were observed between the products and between the storage days. The pH of the products decreased significantly ($P<0.05$) in the following order: Control (6.68-6.75) followed by T1 (6.58-6.63), T2 (6.48-6.56) and T3 (6.46-6.53) incorporated products on 1st day to 30th day of storage. The pH of the products revealed significantly lower ($P<0.05$) pH on 10^{th} day compared to 30th day of storage in Control and treated products had. But no significant differences were found between 1st and 10th day between 1st and 20^{th} day and between 20th and 30th day of storage.

Malav *et al.* (2017) conducted a study to evaluate meat papad prepared using spent hen meat powder and corn flour (Control, T-I), black gram flour (T-II) and combination of corn and black gram flour (T-III).The pH values of control as well as treatment product showed gradual increase with increasing storage period up to 30th day. However, significant ($P<0.05$) decrease was observed on 45th day in control and treatment product.

Cakmak *et al.* (2015) prepared crispy bread snack incorporated with chicken meat and chicken meat powder and evaluated for various physicochemical properties for a period of 3 months. The pH values for the product were decreased during storage and were between 5.840-6.050 at the beginning of storage and between 5.480 and 5.780 at the end of storage. The final pH values were found to be lower than the initial values for all snacks depending on the storage time, but no significant difference was observed between almost all the snack types.

Mishra *et al.* (2015) developed dehydrated chicken meat rings using 90% spent hen meat and found that an acceptable product can be obtained using rice flour with no significant difference in pH value.

Sharma *et al.* (2015) developed a ready-to-eat chicken snack with steamed gelatinized outer case. Utilizing various easily available vegetative extenders like potato (boiled and mashed) and black gram flour, stuff was prepared and the final products were evaluated for. The pH of the product with potato differed significantly ($P>0.05$) from control, though an increase of pH in black gram containing product was non-significant increase.

Kumar *et al.* (2013) developed a product chicken stick using different levels of chicken meat at at 0, 50, 60 and 70% levels. The product recorded a pH value of 6.57-6.91 in chicken sticks with various levels of meat and gram flour and showed a decreasing trend significantly ($P<0.01$) with the increase level of minced chicken meat.

Singh *et al.* (2013) conducted a trial to determine the effects of different levels of baking powder (0, 0.4, 0.5, 0.7, and 0.9%) on the physico-chemical of chicken meat caruncles. The pH of treatment with 0.4% baking powder (6.07) was significantly higher ($P<0.05$) than control (5.95) but there was no significant difference between pH of treatment with 0.5, 0.7 and 0.9% baking powder.

Singh *et al.* (2011) prepared chicken snacks by utilizing spent hen meat and other necessary ingredients and were packaged aerobically as well as under vacuum. They were stored at 30±2°C and analysed for physico-chemical, microbiological and sensory characteristics at a regular interval of 0, 6, 12, 18, 24 and 30 days. The pH was found in increasing order with advancement of the days of storage.

Devalakshmi *et al.* (2010) developed chicken meat chips incorporated with 15% cooked and mashed potato, 15% bengal gram flour and 15% black gram flour which recorded significantly ($P<0.01$) lower pH values than control formulation. The pH values increased during the storage period both at ambient (37+2°C) and refrigerated (7± 1°C) temperature irrespective of type of formulation.

Soni *et al.* (2013) conducted a study to develop dehydrated ready-to-cook meat rings. Mean pH of meat rings was observed to be 6.00, 6.10 and 6.5 respectively in chicken, pork and chevon rings respectively. Chevon rings had significantly higher ($P<0.05$) pH as compared to chicken and pork rings. The average scores of pH of the product indicated a gradually decreasing trend during entire period of storage and significant decrease was observed on 30^{th} day. The pH value of the product on 45th day was significantly lower than initial value but remain comparable with score for 15th and 30^{th} day of storage.

Biswas *et al.* (2015) aimed to standardize the meat level in development of meat wafer using combination of turkey and spent hen meat at the levels of 70, 60 and 50% in the formulation. All the products were evaluated for various physico-chemical and sensory quality characteristics. It has been observed that pH values remain nearly unchanged among different combination products.

2.9.2. Cooking yield

Biswas *et al.* (2015) aimed to standardize the meat level in development of meat wafer using combination of turkey and spent hen meat at the levels following 70% turkey+30% spent hen, 50% turkey and spent hen each and 30% turkey and 70% spent hen. It was found that cooking yield was significantly increased with increase in turkey meat level in combined formulation of meat wafers.

Singh *et al.* (2013) conducted a study to determine the effects of different levels of baking powder (BP) on the physico-chemical and sensory attributes of chicken meat caruncles under 4 different treatment groups namely Control (0%), 0.4, 0.5, 0.7and 0.9% baking powder. The cooking yield per cent of chicken meat caruncles increased significantly ($P<0.05$) with an increase in the level of baking powder.

Nagamallika and Rao (2010) developed chicken meat papads incorporated with vegetable binder and shown that the addition of Bengal gram and black gram flours were effective in chicken meat papad preparation. Papad prepared with 15% black gram flour showed significantly higher cooking yield per cent.

Sharma and Nanda (2002) made efforts to develop chips utilizing spent hen meat. The four formulations were made containing chicken meat at 95, 85, 80 and 75 per cent. Cooking yield of chips in formulation with 95% spent hen meat content showed significantly lower ($P<0.05$) than all other formulations.

Bhoyar *et al.* (1996) prepared reconstructed chicken streaks added with texturized soya protein at different levels of 10, 20, and 30 per cent. They reported that cooking losses were significantly ($P<0.01$) lower in the restructured chicken steaks added with texturized soya protein at 10, 20, and 30 per cent levels than control without texturized soya protein addition.

2.9.3. Tyrosine value

The effect of incorporating 1% drumstick leaf powder (DLP), 1% jamun seed powder (JSP) and 0.5% DLP + 0.5% JSP on physicochemical, sensory and microbiological quality of chicken chips were evaluated during storage at room temperature for a period of 30 days and found no significant differences were observed in tyrosine values among the products throughout the storage study period of 30 days (Kasthuri *et al.*, 2017).

2.9.4. Water activity (a_w)

Modi *et al*. (2007) prepared dehydrated chicken kebab mix with binders and spices and packed in metalised polyester pouches and stored at ambient temperature of 27± 2°C for a period of 6 months. The a_w showed significant increase ($P<0.05$) in values from 0.31% to 0.42% during the study period.

Jaiswal *et al*. (2014) investigated the physico-chemical properties of chicken meat biscuits prepared by replacement of refined wheat flour with different levels (40%, 50% and 60%) of chicken meat powder. The product was stored at ambient temperature (35± 2°C) under aerobic packaging and studied at 0, 10^{th}, 20th and 30^{th} days interval of storage. Water activity values were significantly higher in chicken meat biscuits with increase level of chicken meat powder due to increase water content in meat powder

compared to refined wheat flour. Moreover, significant increased in a_w was also observed during storage period of 30 days under aerobic packaging condition.

Mishra *et al.* (2015) carried out a trial with the objective to develop dehydrated chicken meat rings utilizing spent hen meat along with different extenders adjudged at 10 %, 10%and 5 %for rice flour, barnyardmillet flour and texturized soy granule powder respectively. Water activity of dehydrated chicken meat rings prepared with 10% of rice flour was significantly lower ($P<0.05$) than control but value of a_w for products with 10% level of bamyard millet flour and 5% texturized soy granule protein were comparable with control. Water activity of product with 5% level of texturized soy granule protein was significantly ($P<0.05$) higher than the product with 10% of rice flour, however a_w value for product with optimum level of bamyard millet flour was comparable to other two treatments.

Singh *et al.* (2013) conducted a study to determine the effects of different levels of baking powder on the physico-chemical attributes of chicken meat caruncles. The water activity (a_w) showed no significant difference between control and four treated variants.

2.10. SENSORY EVALUATION

Consumers' acceptance of meat and meat products are determined by certain important factors like its appearance, nutritional value, quality and quantity of final product, price of the product and stability of the product (Miles *et al.* 1984). The bitterness of fenugreek seeds is due to the presence of oil, steroidal saponins, and alkaloids (Singh *et al.* 2022).

Kasthuri *et al.* (2018) developed a ready-to-eat shelf stable functional meat chips with different levels (2, 4 and 6%) of flaxseed powder and (3, 6 and 9%) oats powder. The authors observed that the sensory scores for appearance, flavour, texture, crispiness and acceptability were significantly ($P<0.05$) higher for the product prepared by microwave cooking. Chicken chips containing 4% flaxseed powder and 6% oats powder had scores of 6.37 and 6.47 for appearance; 6.70 and 6.70 for flavour; 6.70 and 6.50 for

texture; 7.0 and 6.83 for crispiness; 6.83 and 6.60 for acceptance, respectively on 8 point hedonic scale.

Nemade and Londhe (2018) undertook a study with a view to standardize the acceptable quality of cookies with incorporation of various levels of spent hen meat powder (0%, 50%, 60% or 70%). The results showed that cookies incorporated with 50% spent hen meat powder exhibited higher scores for all sensory attributes than other treatments.

Kasthuri *et al.* (2017) studied the effect of incorporating 1% drumstick leaf powder, 1% jamun seed powder and 0.5% drumstick leaf powder + 0.5% jamun seed powder on sensory quality of chicken chips were evaluated during storage at room temperature for a period of 30 days. Results on sensory evaluation revealed no significant difference in appearance, flavour, texture, crispiness and acceptability scores between the products and between the storage days All the products had very good sensory score (above 7 in 8 point hedonic scale) throughout the storage study for all the sensory attributes.

Mishra *et al.* (2015) found no significant difference in the appearance scores of treatments both in dried and rehydrated and cooked forms of meat product. Flavour scores for treatments as well as control were comparable among themselves. Texture scores of all the three treatments did not differ significantly with control and among the treatments. Meat flavour intensity scores for products with optimum level of texturized soy granule protein and bamyard millet flour were significantly ($P<0.05$) lower than control however meat flavour intensity score for product with optimum level of rice flour was comparable with control. The overall acceptability scores of control and dehydrated chicken meat rings prepared with different extenders did not differ significantly.

Chand *et al.* (2014) utilized spent hen meat in improving the physicochemical and sensory qualities of dried ready-to-fry chicken meat based chips. It was concluded that formulation containing 60% spent hen meat, 25% Sabudana (Tapioca Pearls) flour, 10% corn flour and 5% potato starch was most acceptable. Sensory evaluation also revealed higher acceptability for chips incorporated with spent hen meat.

Soni *et al.* (2013) conducted a study to develop dehydrated ready-to-cook meat rings from poultry meat and fish and evaluated for various physio-chemical and sensory characteristics. The overall sensory acceptability of poultry meat was found to be significantly higher than fish, whereas moisture is significantly lower. These meat rings retained good acceptability up to 45 days under aerobic condition.

Singh *et al.* (2013) conducted a study to determine the effects of different levels of baking powder (0, 0.4, 0.5, 0.7and 0.9%) on the physic-chemical and sensory attributes of chicken meat caruncles. Chicken meat caruncles prepared using 65% spent hen meat and 0.5% baking powder possessed significantly better scores of sensory attributes along with high cooking yield, moisture content, hardness and crispiness.

Singh *et al.* (2011) prepared chicken snacks by utilizing spent hen meat and other necessary ingredients and were packaged aerobically as well as under vacuum. They were stored at 30±2°C and analyzed for sensory attributes at a regular interval of 0, 6, 12, 18, 24 and 30 days. All the sensory attributes such as colour and appearance, flavour, texture, crispness, aftertaste, meat flavour intensity and overall acceptability indicated decreasing trend during entire storage period at ambient temperature.

Nagamallika and Rao (2010) prepared chicken meat papad with raw and partially cooked spent hen meat with addition of cooked mashed potato, Bengal gram flour and black gram flour each at 15% level and evaluated for quality. Papad with 15% black gram flour showed significantly higher overall acceptability than those with mashed potato, Bengal gram flour and control.

Sharma and Nanda (2002) developed the chicken chips uilizing the spent hen meat. They reported that the sensory scores of color and appearance, meat flavor intensity and overll acceptibility did not decrease significantly from 2 weeks onwards upto 12 weeks while the product ratings still remained good in all the formulations of chicken chips. The products were packaged under nitrogen atmosphere in laminate pouches (aluminium/LDPE) and kept at ambient temperature to assess its shelf life.

Singh *et al.* (2002) concluded from their study that the use of 50% chicken meat was ideal for preparation of chicken snacks with highest score for colour and appearance, texture, crispiness and overall acceptability when compared to control (without meat) and those prepared from 40% and 60% spent hen meat.

Berwal *et al.* (1996) developed turkey meat papads using raw and heat treated (50^0 C for 20 mins) meat by blending with rice flour (50:50) and traditional rice papads were taken as control. It was observed that heat treated turkey meat blended papads had significantly higher sensory scores and better acceptibility.

Meat based snacks can be differentiated on the basis of flavor, taste and hardness from cereal based extruded products (Park *et al.*, 1993).

According to De-Freitas and Molins (1988) sensory quality of meat based snacks was evaluated on the basis of appearance, crispiness, meat flavor intensity, after taste and overall palatability.

2.11. THIOBARBITURIC ACID NUMBER (TBA)

Ahmed *et al.* (2016) analyzed the effect of dried fenugreek leaves at the level of 0, 0.5%, 1.0% and 1.5% in meat burger prepared from mutton and beef during refrigerator storage. Experiment showed decrease in TBA number at 7^{th} and 10^{th} day with increase in fenugreek level.

Kasthuri *et al.* (2017) evaluated the effect of adding 1.0% drumstick leaf powder, 1% jamun seed powder and 0.5% drumstick leaf powder + 0.5% jamun seed powder on TBA value during storage at room temperature for a period of 30 days. It was found that there was no significant differences in TBA values among the products throughout the storage study.

Soni *et al.* (2013) conducted a study to develop a ready-to-cook shelf stable meat rings. The TBARS value increased significantly ($P<0.05$) on 15^{th} day of storage as compared to initial value and thereafter it remain comparable up to 30^{th} day of storage. Whereas non-significant decrease in TBARS value was observed on 45^{th} day of storage.

Singh *et al.* (2011) studied the physico-chemical characteristics of chicken snacks packaged aerobically and vacuum in laminated pouches. The TBA value of chicken snacks initially decreased up to 18 days in vacuum packaging and upto 24th day in aerobic packaging and thereafter increased. The TBA values of aerobically packaged products of 0, 6th and 30th day was significantly ($P<0.05$) different from the product of 12th, 18th and 24th day while vacuum packaged chicken snacks were none significantly different in entire storage period while vacuum packaged chicken snacks were none significantly different in entire storage period.

Jaiswal *et al.* (2014) investigated the physico-chemical properties of chicken meat biscuits prepared by replacement of refined wheat flour with different levels of 40%, 50% and 60%) chicken meat powder. The products were studied on 0, 10^{th}, 20th and 30^{th} days of storage at ambient temperature under aerobic packaging. TBA values for chicken meat biscuits were found to be significantly ($P<0.05$) increased with increased level of meat incorporation due to higher fat in meat as compared to refined wheat flour. TBA values also increased significantly ($P<0.01$) with progression of storage period, but these values were within the acceptable limit of 2.0 mg MDA/Kg of product (Witte et al., 1970) during whole storage period in aerobic packaging condition.

Hollender *et al*. (1987) prepared poultry patties from spent layer meat consisted of 100% breast meat, 50–50 breast and leg meat combination or 100% leg meat. Acceptability scores for both flavour and texture were highest for breast meat patties and lowest for leg meat patties. TBA (2-thiobarbituric acid) values showed increasing values for all treatments with increase in storage period of 3 months.

2.12. ANTIOXIDANT TESTING ASSAY FOR TOTAL PHENOLICS, DPPH, REDUCING ACTIVITY

Fadly *et al.* (2020) in a study aimed to analyze the values of total phenolic content, antioxidant activity, and glycemic values of the non-meat burger patty. There were no significant values for total phenolics between the meat burger and non meat burger.

Mansour and Khalil (2000) evaluated the antioxidant activity of ginger, potato peel and fenugreek seed extracts in ground beef patty. The antioxidant activity was not affected by storage in dark conditions at 5, 25 and 37°C over a period of 21 days, while a significant reduction was observed for extracts kept in light conditions at room temperature (~25°C).

2.13. ANTIMICROBIAL TESTING ASSAY AGAINST *E. coli, Salmonella, Staphylococcus*

Total microbial contamination level of poultry meat and meat product depend on initial quality and processing conditions (Panov and Labyanestski, 1979).

Kasthuri *et al.* (2017) analyzed the effect of incorporating 1% drumstick leaf powder, 1% jamun seed powder and 0.5% drumstick leaf powder + 0.5% jamun seed powder on the microbiological quality of chicken chips during storage at room temperature for a period of 30 days. Storage had no significant effects on standard plate count (SPC) up to 20 days of storage and on yeast and mould count (YMC) up to 30 days of storage.

Soni *et al.* (2013) found the average total plate count (log cfu/g) of the dehydrated meat rings to be in increasing trend from the initial values of 2.93 ±0.02 to 5.18 ±0.02 after 45 days during storage of the product. No coliforms were detected throughout the storage study. Yeast and moulds were not detected on 0 day of ambient storage but increased significantly on 15th day and thereafter remain stable upto 30^{th} day of storage and again increased significantly on 45^{th} day. The total bacterial and coliform group counts not exceed 5 and 3 log cfu /g, respectively in beef burgers (E.O.S., 2005)

Singh *et al.* (2011) prepared chicken snacks by utilizing spent hen meat and other necessary ingredients and were packaged aerobically and under vacuum. The products were stored at 30±2°C and analyzed for microbiological characteristics at a regular interval of 0, 6, 12, 18, 24 and 30 days. The Total Plate Count (TPC) (cfu/g) of the products, irrespective of its packaging type indicated an increasing trend during storage after 6th day of storage and increased significantly ($P<0.05$) after every 6 days till 30^{th}

day of storage. *E Coli* count (cfu/g) of the products in both the packaging system was not detected till 18th day, after that it indicated an increasing trend. The *E Coli* count in the products during storage differed significantly ($P<0.05$) to each other from 24th to 30th day. Yeast and Mold Count (cfu/g) in chicken snacks was also not detected till 18th day; after that an increasing trend in yeast and mould count was noticed during the entire period of storage. Like Coliform, Yeast and mould count of the product in both packaging systems increased significantly ($P<0.05$) after 24th day. Higher count for total plate count, Coliform and Yeast mould were noticed in aerobically packaged products as compared to vacuum packaged products.

Sharma and Nanda (2002) developed chips utilizing meat from spent hen at 95, 85, 80 and 75 per cent, respectively. During the storage period, total aerobic count and yeast and mould counts remained well below the permissible limits.

Hegazy (2011) aimed to evaluate the effect of using fenugreek seed flour (at the rate of 3, 6, 9, and 12%) in meat products on the microbiological characteristics of beef burger patties during frozen storage. Results showed that with increase in addition of level of fenugreek seed flour indicated decrease in total bacterial count, psychrophilic count, coliform bacterial count at frozen temperature.

Das and Jayaraman (2003) prepared chicken pulav from chicken meat and stored under ambient and chill temperature. The findings reported absence of coliforms during ambient temperature storage of dehydrated chicken pulav which aids in increasing the shelf life of the product up to 18 months.

Modi *et al.* (2007) prepared dehydrated chicken kebab mix with binders and spices. The chicken kabab mix were packed in metalised polyester pouches and stored at ambient temperature (27± 2°C) for 6 months and sampled periodically for quality evaluation. The chicken kebab mix was microbiologically safe as indicated by low bacterial counts and absence of coliforms throughout the storage period of 6 months.

2.14. COLOUR PROFILE ANALYSIS

Mishra *et al.* (2015) found no significant difference in the redness value between control and treatments as well as among the treatments. Yellowness value of dehydrated chicken meat rings prepared incorporating texturized soy granule proten was significantly lower (P <0.05) than control. Product with optimum level of rice flour and bamyard millet flour had non-significantly lower yellowness value than control but had significantly higher (P <0.05) value than product with level of texturized soy granule proten.

Singh *et al.* (2013) analyzed for the colour profile of chicken meat caruncles containing 0, 0.4, 0.5, 0.7 and 0.9 % baking powder, where *L* values ranged from 24.30-38.29 and were found to be non-significant although it remained slightly higher in all the treated groups than the control group. The *a* value of control, treatment groups with 0.5 and 0.9% baking powder did not differ significantly among themselves. The *b* value was also non-significant between different groups.

Wagh *et al.* (2015) studied the effect on pork frankfurters with the incorporation of 0.30% sea buckthorn, 0.10% grape seed, 0.03% green tea , 0.12% fenugreek seed and 0.10% *Acacia catechu* during 20 days of refrigerated aerobic storage. In samples with sea buckthorn and *Acacia catechu* exhibited an increased redness producing higher *a** values than other treatments. However, treatment with green tea was more effective in increasing *b** values than other treatments at the end of storage

Popped cereal snacks using various combination of spent hen meat and grains (potato starch, corn starch, and rice flour) were developed. Popped snacks with grains only were higher in L* value than those with meat and grains. The a* and b* values increased with increasing meat content. (Lee *et al.*, 2003)

2.15. CHOLESTEROL LEVEL

Mishra *et al.* (2015) researched with the objective to develop dehydrated chicken meat rings utilizing spent hen meat and different extenders. The total cholesterol content (mg/g) for products with optimum level of rice flour and bamyard millet flour were

significantly lower (P<0.05) than control. The total cholesterol content was comparable and products with optimum level of rice flour and bamyard millet flour as well as in products with optimum level of texturized soy granule protein and bamyard millet flour. The cholesterol content for products with optimum level of texturized soy granule protein was comparable with control but was significantly higher (P<0.05) than products with optimum level of rice flour.

Indumathi *et al.* (2019) prepared sausages made from spent broiler breeder hen meat and compared with sausages made from broiler on the basis of certain physicochemical, cholesterol and sensory evaluation. The study recorded significantly (P<0.01) lower cholesterol values in sausages made from spent hen meat when compared to the sausages made from broiler.

2.16. STORAGE STABILITY

Nemade *et al.* (2021) developed cookies utilizing spent hen meat powder replacing with pearl millet flour. The products were stored at ambient temperature (37±10C) for a storage period upto 60 days. it revealed that the prepared cookies were well accepted with incorporation of 50% spent hen meat powder and safely stored in LDPE upto 60 days at ambient temperature (37 ± 10C) without adversely affecting its quality.

Kasthuri *et al.* (2017) found that functional chicken chips with drumstick leaves powder (1%), jamun seed powder (1%) and drumstick leaves powder (0.5%) + jamun seed powder (0.5%) could be preserved under aerobic condition for 30 days without any substantial drop in qualities.

Devatkal *et al.* (2012) carried out to evaluate the antioxidant properties and antioxidant effect of 2% water extracts from powders of curry leaves and fenugreek leaves in ground chicken patties stored aerobically for 8 days at refrigerated temperature 4±1°C. there was significant decrease in TBARS values in products containing fenugreek and curry leaves extract than the control group during storage period.

Singh *et al.* (2009) prepared chicken snacks using spent hen meat packaged aerobically in laminated pouches and stored at 30 ± 2^0C and analyzed for physic chemical microbiological and sensory characteristics for a period of 30 days and found not much change in the contents of different constituents, microbiological parameters and sensory profile was noticed.

Das and Jayaraman (2003) developed a dehydrated chicken pulav from chicken meat and preserved under ambient and chill temperature in pouches of polypropylene film (PP) and paper-foil-polyethylene laminate (PFP). The study proved that the product can be effectively preserved with a shelf-life of 8-12 and 14-18 months under ambient and chill temperature, respectively.

2.17. COST OF PRODUCTION

Raman *et al.* (2020) intended to standardize processing protocol of chevon patties with the incorporation of egg albumin, skim milk powder and with different antioxidants and to evaluate cost of production of final developed product. Three treatments were prepared with the incorporation of jamun seed extract (T1), fenugreek seed extract (T2) and their 1:1 combination (T3) in products by replacing of meat (1%) from formulation to evaluate economics of chevon patties. In the cost economics, cost of formulation was found highest for group T1. The break-even point was estimated as Rs 239413.75for control while Rs 238713.75, Rs 239093.75 and Rs 238903.75 for T1, T2 and T3 respectively. The cost benefit ratio was found highest for control and lowest for T1. The estimated details of economics of the developed product concluded that viable enterprises can be established by keeping rate Rs 437 for control and Rs 443, Rs 439, Rs 438 for jamun seed, fenugreek seed, their 1:1 combination incorporated products respectively.

Nemade and Londhe (2018) undertook a study with a view to standardize the acceptable quality of cookies with incorporation of various levels of spent hen meat powder (0%, 50%, 60% and 70%). Cost of production revealed that cost effective cookies were prepared with incorporation of 50% spent hen meat powder as compareto addition of 60% and 70% SHMP. The overall cost for the production of 1kg control

cookies was Rs. 98.46/-and 50% incorporated spent hen meat powder cookies was Rs. 110.96 /-. Similarly, the overall cost of production for 1kg cookies incorporated with 60% and 70% was Rs. 113.49/-, Rs. 115.98/- respectively. Thus, we can say that cookies incorporated with 50% spent hen meat powder had lower cost of production than cookies incorporated with 60% and 70% spent hen meat powder.

Singh *et al.* (2016) in a study used broccoli and carrot powder in development of meat cutlets their quality and storage stability was evaluated along with cost of production. The total production cost of developed meat cutlets (per 100 kg) was calculated on the basis of cost of formulation, breading, frying and overhead production which was Rs. 10471.00, Rs. 2567.00, Rs. 255.00 and Rs. 2286.00, respectively. The production cost was calculated on the basis of cooking yield and recorded as Rs. 176 for control and carrot powder incorporated cutlets and Rs. 181 for broccoli powder incorporated meat cutlets. Total profit from sale of control meat cutlets is around Rs. 56/kg and the profit per month were found to be Rs. 140000. Net profit per month was found to be Rs. 93334 after repayment of loan amount Rs. 46666 per month for 12 months. Break Even Point was estimated around 12 and 273602 on the basis of unit and rupee sales, respectively. Cost benefit ratio was around 0.35 and return on investment was 35%.

Berwal *et al.* (2013) concluded that the cookies with 10 per cent chicken meat mince and wheat flour would provide a nutritious with sufficient amount of essential amino acids (lysine, tryptophan and threonine), convenient and ready-to-eat food item. The cost of production of cookies with 10 per cent chicken meat mince worked out to be Rs. 77.60 per kg.

CHAPTER - III

Materials and Methods

DEVELOPMENT OF READY-TO-COOK CHICKEN CHIPS USING SPENT HEN MEAT INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

CHAPTER-III

MATERIALS AND METHODS

3.1. LOCATION OF WORK

The study was conducted at the Department of Poultry Science, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati 781022.

3.2. PREPARATION OF POWDER OF CRUDE FENUGREEK LEAVES AND SEEDS

Fenugreek (*Trigonella foenum graecum*) seeds were purchased from local market of Guwahati city. The seed were cleaned thoroughly under running water and dried at 55-60°C for 2 hours (Qureshi *et al*., 2018). The seeds were ground into fine powder in an electric grinder and sealed hermetically and stored in plastic containers for further use.

Fresh and healthy leaves were collected from local source of Guwahati. The leaves were thoroughly washed to remove the undesired particles and dried it as fast as possible to prevent fungal infection and preserve its natural green color. Accordingly, leaves were dried in an oven at 40 °C for two days (Ahmad *et al*. 2018). The dried leaves were crushed into fine powder using an electric grinder, sealed and stored in dark containers at 4° C.

3.3. PROXIMATE ANALYSIS OF FENUGREEK SEED AND LEAVES WERE DONE AS PER THE AOAC (1970)

3.3.1. Moisture

A total of 10g of product sample were weighed in moisture cups and kept for drying in hot air oven at 80^{o} C for 16 hrs with the lids slightly open. After completion of drying, the cups were removed and placed in a desiccator to cool down. The moisture cups with the dried sample was measured and again transferred to hot air oven. The process was repeated until constant weight of the sample was obtained. The percentage of moisture was calculated by the following formula:

$$\text{Moisture (\%)} = \frac{\text{Weight of moisture cup with Sample – weight of moisture cup with dried sample}}{\text{Weight of moisture cup with Sample – weight of empty moisture cup}} \times 100$$

3.3.2. Crude protein

It was determined by Micro Kjeldahl method. For this, 0.2g of samples was digested in the Kjeldahl flask with 10ml concentrated H_2SO_4 and 3g of digestion mixture in the digestion unit of KEL PLUS KES 6L (Make: Pelican Equipment). The temperature was maintained at 400^0 C and the samples were boiled for 2 ½ hrs until the solution turns into clear green. The digested samples were allowed to cool to room temperature and distilled in the distillation unit .The distillate containing methy red was titrated against 0.1 N HCl. From the titre of each sample the crude protein content was calculated by using the following formula:

$$\text{Crude protein (\%)} = \frac{\text{14 x Normality of the acid x Titration value x 6.25}}{\text{Weight of the sample x 1000}} \times 100$$

3.3.3. Ether extract

1gm of each dried samples was weighed and put into thimble and put inside the oil flasks of the SOCS INFRA SIS 6 Ether Extractor (Make Pelican Equipment). Petroleum ether (60-80%) was poured into the upper chamber of the extractor and allowed to come down to the oil flasks. The temperature was fixed at 95^o C for initial 1 hour and thereafter raised to 170^o C to 180^o C. Ether was collected at the upper chamber of the extractor. The oil flasks containing extract at the bottom were subsequently transferred to a hot air oven to evaporate the residual ether. Oil flasks were then cooled in desiccator and weighed.

$$\text{Ether Extract (\%)} = \frac{\text{Weight of oil flask with extract – Weight of oil flask}}{\text{Weight of the sample}} \times 100$$

3.3.4. Total Ash

2g of sample was taken in a clean dry silica crucible after weighing. The crucible was placed over electric burner to burn out the sample till the smoke stops. Now the crucible was placed in a muffle furnace to burn the sample at 600 ^{0}C for 2 hours. At this temperature all organic matter will be burnt leaving behind the minerals. The crucible was removed from the furnace carefully and cooled down in a desiccator and weighed.

$$\text{Ash content (\%)} = \frac{\text{Weight of crucible with sample after complete ashing – Weight of empty crucible}}{\text{Weight of the crucible with sample - Weight of empty crucible}} \times 100$$

3.4. ANTIOXIDANT ESTIMATION OF FENUGREEK LEAVES AND SEEDS WAS DONE AS PER THE METHOD DESCRIBED BY HWA *et al.* (2019) WITH PARTIAL MODIFICATIONS

3.4.1 Preparation of extract of fenugreek leaves and seeds

500g of seeds of *Trigonella foenum-graecum* (Fenugreek) were weighed and allowed to dry under room temperature for a duration of one week. The seeds were placed under shade without exposing to the sunlight to avoid any evaporation of active constituent. The seeds were crushed into powder by using the grinder and collected in the conical flasks.

250g of seeds powder were weighed and added in 500ml of ethanol (95%). The mouth of the conical flask was covered with aluminium foil to avoid any evaporation of the ethanol and the active components of the extract. The flask was then kept in dark in a cupboard for one week. The mixture was filtered by using muslin cloth. The filtrate was collected in a conical flask and kept covered with aluminium foil.

The filtrate was allowed for evaporation by using hot waterbath at temperature 63 C for approximately 4 hours. The extracts were transferred to the conical flasks and the mouth of conical flasks was sealed with aluminum foil.

3.4.2 DPPH radical scavenging activity (Soni and Sosa, 2013)

The DPPH radical scavenging activity was measured according to the method of Soni and Sosa (2013) with some modifications. The Soxhlet extract was used in antioxidant activity screening. Four different concentrations of extracts were prepared at 50, 100, 150 and 200µg/ml. Ascorbic acid was used as standard. 3ml of 0.1mM of methanolic solution of DPPH was added to 2.5ml of different concentration of plant extracts. The tubes containing the mixtures were covered with aluminum foil. The mixture was kept in the dark place at 37^{o} C for 30 minutes. Then, the absorbance was measured at 518 nm using a spectrophotometer. The same procedure was repeated by replacing the extracts with different concentration of BHT to determine the standard graph. The control absorbance value was determined by repeating the same procedure with replacement of the extracts with ml of ethanol. Sample measurement was done once and the result was calculated. The anti-oxidant activity was determined by following formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

3.4.3. Total phenolics content (Singleton *et al.*, 1999)

For this method four different concentrations of extracts were prepared which were 25µg/ml, 50µg/ml, 100µg/ml, and 200µg/ml. Gallic acid was used as the standard for this assay. For every 0.2ml of extract, about 0.2ml of folin-ciocalteu reagent and 4ml of 2.5% sodium carbonate were added. The compounds were blended thoroughly and allowed to stand for 2 hours. The absorbance of the solution at 750nm was measured using spectrophotometer. The same procedure was repeated by replacing the extract with the gallic acid. Quantification of total phenolic content was done using standard curve of gallic acid as a standard phenolic compound (1, 2, 4, 6, 8 and 10µg/ml), which was dissolved in ethanol and expressed as mg gallic acid per gram of plant mater.

3.4.4. Reducing activity (Jayprakash *et al*., 2001)

The Reducting Activity was measured according to the method outlined by Jayprakash *et al.* (2001). Different concentration of sample extracts (100, 200,300,400, µg/ml) one ml each were mixed with 2.5 ml of 1% Potassium ferricyanide and 2.5ml of phosphate buffer having pH 6.6. This mixture was incubated at 50^{o} C for 20 minutes. Then 2.5ml 1% Trichloroacetic Acid (TCA) was added and centrifuged at 3000 rpm for 10 minutes. From the solution, 2.5ml of supernatant was taken. To this 2.5ml of solution, 0.5ml of ferric chloride was added. Absorbance was measured at 700 nm by using Spectrophotometer. Butylated Hydroxy Toluene (BHT) was taken as standard here.

3.5. ANTIMICROBIAL TESTING (against *E coli, Salmonella gallinarum, Staphylococcus aureus*)

For the antimicrobial activity of the plant extract, agar well diffusion method was used as described by Baydoun *et al.,* (2017) with slight modification as given below.

3.5.1 Agar well diffusion method (Baydoun *et al.*, 2017)

Agar well diffusion method was widely used to evaluate the antibacterial activity of plants extracts. The Muller Hilton Agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8mm was punched aseptically with the back of a sterile microtip, and a volume (50µL) of the antimicrobial agent or extract solution at desired concentration (100, 200, 300 mg/ml) was introduced into the well. Then, agar plates were incubated under at temperature of 37°C for 24 hours. The antimicrobial activity of the fenugreek seeds and leaves were evaluated against both Gram positive and Gram negative bacteria. The pathogenic microorganisms used in this study were: *E coli* (ATCC 6633), *Staphylococcus aureus* (ATCC 6633) and *Salmonella* typhimurium (MTCC-98) strains were sub-cultured from the repository pure culture of Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. All of the processes of antimicrobial screening activity were done in laminar air flow cabinet to avoid any contamination or cross contamination might occur especially during the process of nutrient agar preparation and well diffusion test. The zone of

inhibition of each plate was observed, measured and recorded in millimeters (mm) for positive standard, antibiotic discs of Ampicillin (10mcg), Chloramphenicol (30mcg) and Tetracycline (5mcg) were taken. Ethanol was taken as the negative control.

3.6. QUALITATIVE PHYTOCHEMICAL ANALYSIS OF FENUGREEK SEEDS AND LEAVES

The qualitative phytochemical analysis of both the fenugreek seeds and leaves were carried out to ascertain the process of some active constituents in the seeds and leaves using standard protocols (Swain and Hills, 1959)

3.6.1 Salwoki test for steroids (Swain and Hills, 1959)

Five mg of aqueous extract was dissolved in 3ml of chloroform and then shaken with 3ml of concentrated sulphuric acid. Development of red colour indicates the presence of steroids.

3.6.2.Test for phenolic compounds (Methaq *et al*., 2017)

Five mg of the aqueous extract was dissolved in 1ml of water and 5 drops of 10% ferric chloride was added to it. Development of dark blue colour indicates the presence of phenolic compounds.

3.6.3.Test for tannins (Swain, 1979)

Three ml of 1% ferric chloride solution was mixed with 0.002g of aqueous extract. Development of blue green or brownish colour indicates the presence of tannins.

3.6.4.Test for saponin (Kumari e*t al.,* 2016)

Two grams of the samples were boiled with 20ml of distilled water in a hot water bath and filtered through Whatman paper No. 1. Then 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously to form a stable and persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion was considered to be an indication of presence of saponin.

3.6.5.Test for flavanoids (Harbone, 1973)

To 2ml of methanolic solution of aqueous extract (0.5g in 10ml methanol), few drops of neutral ferric chloride solution was mixed. Development of green colour indicates the presence of flavanoids.

3.6.6.Test for alkaloids

Two ml of aqueous sample extract was measured using a measuring cylinder and equal volume of ethanol containing 3% tartaric acid was added and shaken. A few drops of marquin's reagent were added into the mixture. The formation of precipitate indicates the presence of alkaloids.

3.7. PRELIMINARY TRIALS

For the present study, a total of 20 live healthy spent chickens were procured from the Instructional Poultry Farm, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati. The birds were slaughtered in the Department of Poultry Science as per standard procedure. Then the meat was harvested, washed thoroughly after deboning and then kept at 24 hours (4 ± 1^0C).

A series of preliminary trials were conducted to standardize the basic formulation for preparation of the chicken meat chips from spent hen. Different processes and formulations to prepare an acceptable quality ready-to-cook chicken chip were tried and finally the basic formulation and processing methodology as mentioned was adopted on the preliminary findings.

3.8. OPTIMIZATION OF THE LEVELS OF NON-MEAT INGREDIENTS

In this experiment, different extenders such Bengal Gram flour, refined wheat flour were incorporated separately into the basic formulation of chicken eat chips (standardized on the basis of preliminary trials), replacing the lean meat.

Standard procedures were followed to prepare the product. The product was standardized first with best suitable formulation with suitable level of ingredients.

Fenugreek seeds and fenugreek leaves at different levels were added to study the antioxidant and shelf life of the product under ambient temperature for a period of 30 days. Treatment groups are made with incorporation of the following levels of Fenugreek seed powder and Fenugreek leaves powder replacing lean meat in formulation of the product.

The final formulation for chicken chips was determined after pilot study of the product with the following ingredients and composition.

TABLE 3.1: STANDARD FORMULATION FOR CHICKEN CHIPS

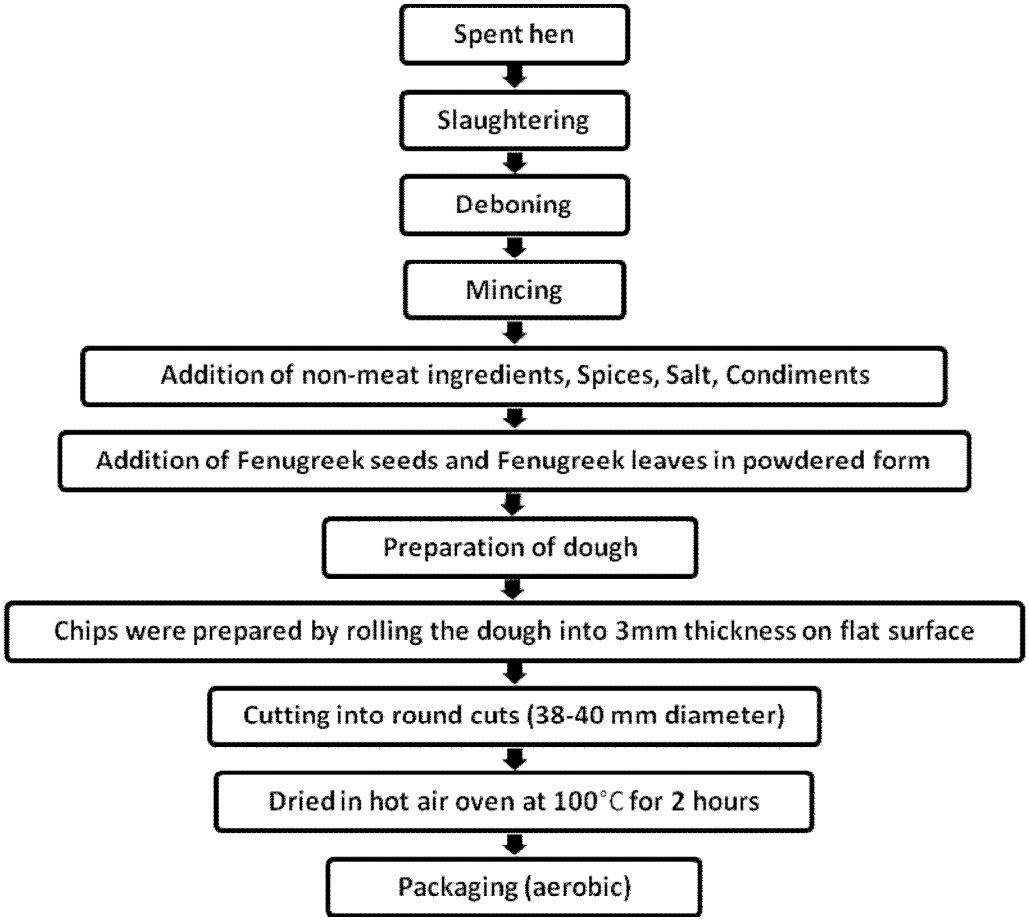
INGREDIENTS	PARTS
Meat	60.0
Wheat Flour	10.0
Bengal Gram Flour	12.0
Baking powder	0.5
Corn flour	12.0
Salt	2.0
Spices*	1.5
Condiments (ginger & garlic)**	2.0
Total	**100**

***Spice mix formulation :** The spices and their composition used in this study are given in the Appendix I. cleaned spics without extraneous materials were dried and ground to powder, sieved to obtain fine powder, which was sealed hermetically stored in refrigerator for subsequent use.

****Condiments mix :** Condiments used in this preparation were garlic and ginger in the ratio of 2:1. Their external coverings were peeled off and made into fine paste using grinder.

3.9. PROCEDURE FOR CHIP PREPARATION

The chips were prepared as per the procedure developed by Devalakshmi *et al.* 2010 with slight modifications.



The product was standardized with best suitable formulation with suitable level of ingredients. Fenugreek seeds and leaves at different levels were added to study the antioxidant and shelf life of the product under ambient temperature. Treatment groups are made with incorporation of the following levels of fenugreek seed powder and fenugreek leaves powder replacing lean meat in formulation of the product. After the Phase I trial, four treatment groups were selected on the basis of organoleptic tests, physicochemical parameters such as pH, water activity, cooking yield, tyrosine value and were taken for further studies.

TABLE 3.2: FORMULATION FOR PREPARATION OF CHICKEN CHIPS INCORPORATED WITH FENUGREEK LEAVES AND/OR SEED POWDER

INGREDIENTS	PARTS						
	Control	**T -I**	**T-II**	**T-III**	**T-IV**	**T-V**	**T-VI**
Meat	60.0	59.75	59.50	59.00	59.75	59.50	59.00
Wheat Flour	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Bengal Gram Flour	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Baking powder	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Corn flour	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Salt	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Spices	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Condiments	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fenugreek leaves	0	**0.25**	**0.50**	**1.00**	0	0	0
Fenugreek seeds	0	0	0	0	**0.25**	**0.50**	**1.00**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**

3.10. PERIOD OF THE STUDY:

The research trial was conducted for a period of 30 days.

3.11. PARAMETERS UNDER STUDY-PHASE I-

Parameters pertaining to different attributes recorded throughout the experiment period.

3.11.1. Proximate Analysis of the Treatment groups

The percent moisture, crude protein, ether extract, crude fibre and total ash were estimated as per methods of AOAC, 1970.

3.11.2. Physicochemical parameters

3.11.2.i) pH:

The hydrogen ion concentration of the dry chicken chip were determined by following the method as described by Trout *et al*. (1992) by using a digital pH meter (model). 15g of sample was homogenized with 30ml distilled water in a mechanical laboratory stirrer (model) and the homogenate was filtered through Whatman No. 1 filter paper. The filtrate obtained was used for measurement of pH in the digital pH meter. The pH values were recorded on the day of processing and at 10 days interval up to storage period of 30 days.

3.11.2.ii) Cooking yield

Cooking yield (%) was calculated and expressed as percentage by the following formula, (Malav *et al.*, 2017)

$$\% \text{ Cooking yield} = \frac{\text{Weight of microwave chips}}{\text{Weight before cooking}} \times 100$$

The cooking loss of the samples was determined on the first day of the experiment.

3.11.2.iii) Tyrosine value

Tyrosine value is determined by a modified method of Pearson (1968) as described by Strange *et al*., (1977).

Preparation of Trichloroacetic acid extract:

Twenty (20) grams of meat sample was blended in a mincer with 50 ml of cold 20% trichloro acetic acid for 2 minutes. The blended content was rinsed in 50 ml

distilled water. It is mixed together and filtered through the Whatman No.1 filter paper (18.5 cm diameter.) and the volume of the filtrate is collected in a 100 ml measuring cylinder. The filtrate is known as trichloroacetic acid extract.

Two and half ml (2.5 ml) of trichloroacetic acid extract was diluted with equal quantity of distilled water is a test tube. To this, 10 ml of 0.5 N Sodium hydroxide is added followed by 3 ml of diluted folinciocalteu phenol reagent (1 part of folinciocalteu phenol reagent: 2 parts distilled water). After mixing and keeping it for 15 minutes at room temperature the developed blue color is measured as absorbance at 660 nm in a spectrophotometer using a blank for comparison. With reference to the standard graph the tyrosine value will be calculated and expressed as milligram of tyrosine/100 gm of meat sample.

Preparation of standard graph for estimation of tyrosine value:

One hundred milligrams of pure tyrosine is dissolved in 500 ml of 5% trichloroacetic acid in a volumetric flask. The following volumes of tyrosine solution are then transferred to a series of 100 ml volumetric flasks i.e., 0 ml, 1 ml, 3 ml, 5 ml, 7 ml, 9 ml, 10 ml, 12 ml, 15 ml, and 20 ml. The volume of each volumetric flask is made upto the mark with distilled water and mixed thoroughly. To 5 ml of each of this solution in a test tube, add 10 ml of 0.5 N sodium hydroxide and 3 ml of diluted folinciocalteu phenol reagent and mixed well. These are kept for 15 minutes at room temperature. The developed color is measured as absorbance at 660 nm in a spectrophotometer as described earlier. The values of absorbance recorded for various dilutions of tyrosine are plotted on the graph sheet.

3.11.2.iv) Water activity (a_w):

Water activity was measured with the help of a water activity meter. Ground samples was taken in the sample container of the water activity meter (model) and introduced inside the meter, closed the upper lid and pressed the button and the reading was recorded.

3.11.3. Sensory evaluation

Sensory evaluation of control and treatment spent hen meat chips were performed, utilizing an eight point hedonic score card (Keeton, 1983) with slight modifications, where 8=excellent and 1=extremely poor. The pretrained sensory panelists consisted of faculty and postgraduate students of the Department of Poultry Science and Department of Livestock Products Technology, College of Veterinary Science. AAU. Chips were cooked and served immediately to the panelists. The panelists evaluated the samples for attributes such as colour, flavor, crispiness, texture, after taste, and overall acceptability.

TABLE 3.3: HEDONIC SCORE CARD (Keeton, 1983)

SCORE UNDER HEDONIC SCALE	
EXCELLENT	7
VERY GOOD	6
GOOD	5
FAIR	4
POOR	3
VERY POOR	2
EXTREMELY POOR	1

3.12. PARAMETERS UNDER STUDY -PHASE II

Based on the analytic values of the treatment groups from the Phase I experiment, the best of the treatment groups each with a particular level of fenugreek seed and leaves powder were selected.

TABLE 3.4: FORMULATION FOR PREPARATION OF CHICKEN CHIPS INCORPORATED WITH FENUGREEK LEAVES AND/OR SEED POWDER

INGREDIENTS	PARTS			
	Control	**Treatment A (with 0.25% FL)**	**Treatment B (With 0.25% FS)**	**Treatment C (0.25% FL+FS)**
Meat	60.0	59.75	59.75	59.50
Wheat Flour	10.00	10.00	10.00	10.00
Bengal Gram Flour	12.00	12.00	12.00	12.00
Baking powder	0.50	0.50	0.50	0.50
Corn flour	12.00	12.00	12.00	12.00
Salt	2.00	2.00	2.00	2.00
Spices*	1.50	1.50	1.50	1.50
Condiments**	2.00	2.00	2.00	2.00
Fenugreek leaves	0	**0.25**	0	**0.25**
Fenugreek seeds	0	0	**0.25**	**0.25**
Total	**100**	**100**	**100**	**100**

3.12.1. Proximate analysis of the Treatment groups

The percent moisture, crude protein, ether extract, crude fibre and total ash were estimated as per methods of AOAC, (1970).

3.12.2 Physicochemical parameters

3.12.2.i) pH

The hydrogen ion concentration of the dry chicken chip was determined by following the method as described by Trout *et al.* (1992) by using a digital pH meter.

3.12.2.ii) Tyrosine value

Tyrosine value was determined by a modified method of Pearson (1968) as described by Strange *et al*., (1977).

3.12.2.iii) Water activity (a_w)

Water activity was measured with the help of a water activity meter. Ground samples was taken in the sample container of the water activity meter and introduced inside the meter, closed the upper lid and pressed the button and the reading was recorded.

3.12.2.iv) Cooking Yield

The cooking loss of the chicken chips was determined from the difference in weights of dried chicken chips and the cooked chips samples with following formula:

$$\% \text{ Cooking loss} = \frac{\text{Weight of dried chips} - \text{Weight of cooked chips}}{\text{Weight of dried chips}} \times 100$$

The cooking loss of the samples was determined on the first day of the experiment.

3.12.3 Storage stability under aerobic packaging up-to 30 days at 10 days interval

3.12.3.i) Thiobarbituric acid number

The TBA values of the samples were determined at different periods of storage by the method as described by Witte *et al*. (1970).

10g of sample was blended for 15minute in a mechanical blender with 25ml of extracting solution (4°C) containing 20 per cent trichloroacetic acid in 2M orthophosphoric acid. The resulting solution was transferred quantitatively into a 50ml volumetric flask and made up to 50ml with distilled water and mixed thoroughly. A 25 ml of the solution was filtered through Whatmann No1 filter paper. 5ml of the filtrate was transferred to a screw capped test tube followed by addition of 5ml 2-thiobarbituric acid (0.05M in distilled water). The tube was tightly capped and solution was mixed by inversion and kept in dark for 12-14 hr at room temperature. The optical density of the resulting solution was measured in a Systronics Spectrophotometer 106 at 530 nm. The

TBA value was calculated by multiplying the absorbance by the K value (5.2) for extraction and expressed as mg malanoldehyde per kg of chips.

3.12.4. Assay of total cholesterol

Cholesterol content of freshly prepared chicken chips was determined using cholesterol test kit except that instead of blood serum, lipid extract was used. Lipid extract was prepared by taking one gram of ground chips sample and adding 10 ml of freshly prepared 2:1 chloroform: methanol solution and homogenized. Homogenate was filtered using Whatman No. 42 filter paper and to 5 ml of filtrate equal quantity of distilled water was added, mixed and centrifuged at 3,000 rpm for 7 min. Top layer (methanol) was removed by suction. Volume of the bottom (chloroform) layer having cholesterol was recorded. From this, 25 µl of the sample was pipetted in a test tube and kept in water bath (100°C) for 2-3 minutes till it dried. To this 5 ml of cholesterol reagent was added, mixed and kept in a boiling water bath for 90 seconds for colour development. The O.D. of standard and test against blank (5ml chloroform) was taken at 530 nm in a spectrophotomete and expressed as mg per g of sample.

3.12.5. Color profile analysis

Color measurements were taken using a colorimeter Color evaluation (l*, a* and b*) was measured on the surface of the chicken chips samples with results taken in triplicate for each sample and the results are expressed as mean.

3.12.6. Antioxidant testing assays (Total phenolics, DPPH)

3.12.6.i) Total Phenolic Content Analysis

The total phenolic content determination was executed by folinciocalteu colorimetric method (Singleton *et al.*, 1999). This method involved preparation of a blank solution and gallic acid as a standard solution. A blank solution was prepared by means of 2ml 96% ethanol into a 10 ml test tube. Gallic acid solution was prepared by making a stock solution of 800 ppm concentration in 100 ml. Then, 10 mg of gallic acid was dissolved in 50 ml 96% ethanol in an extract bottle of 60 ml. Then dilution was carried out with concentrations of 0, 50, 100, 150 an 200 µg/ml at a volume of 2 ml.

Sample preparation was initially prepared by homogenization. A total of 0.5ml of samples was extracted with 5ml methanol, stored at room temperature for 2 hours in the dark. The sample was then centrifuged. The supernatant extract was then used for analysis. 25µl extract was oxidized with folin-ciocalteu reagent, and the reaction was neutralized with sodium carbonate for 60 minutes at room temperature. Then the absorption at 760 nm was measured using Systronics Spectrophotometer 106.. The total value of phenol was interpreted in milligrams equivalent to gallic acid per gram of extract (mg GAE/g extract). The determination of the GAE mg/g value was based on calculations from the simple linear regression equation of the gallic acid standard curve. The total phenolic calculation was as follows

$$\text{Mg GAE/g} = \frac{\text{Sample concentration (mg/l) x total volume of the test solution}}{\text{Weight of the extract (g) x 1000}}$$

3.12.6.ii) DPPH radical scavenging activity

Antioxidant activity was determined by the DPPH method (Mollyneux, 2004). The sample was diluted into methanol (1 mg/ml). In a total volume of 1 ml, a test solution consisting of 500 µl of sample and 500 µl DPPH (125 µM in ethanol) was added to the test solution. The test sample solution was dissolved and then allowed to stand at room temperature and dark for 30 minutes. Then, the absorbance was measured at 517 nm using a Systronics Spectrophotometer 106.

$$\text{Inhibition (\%)} = \frac{\text{Absorption of control} - \text{Absorption of sample}}{\text{Absorption of sample}} \text{ x } 1000$$

3.12.7. Microbiological studies

3.12.7. i) Total plate count

Enumeration of the total viable plate count of the chicken chips samples were done in standard plate count agar medium, pH 7.0±0.1 and the count was made at

different storage period by the pour plate technique as described by Harrigan and McCance (1976). The plates were incubated for upto 72 hours at 37^{o} C.

A total of 10g of samples of chicken chips was blended with 90ml of sterile normal saline solution in a homogenizer to give 10^{-1} dilution. Ten fold serial dilutions were made and from the appropriate dilution, 1 ml of the innoculum was transferred to petridish, poured with standard plate count agar and mixed properly by 5 times to and fro, 5 times clockwise, 5 times to and fro at right angles to the first and 5 times anticlockwise. Plates were incubated at 37^{o} C up to 72 hours for enumeration of total viable aerobic counts. Counting was done by using a bacteriological colony counter and all those plates yielding >25 and < 250 bacterial colonies were taken into account.

3.12.7.ii) Test for Coliform bacteria

The collection, preparation of samples, dilution and plating for the determination of Coliform count was similar to that of the above stops followed in the estimation of Total Plate Count. The plating media used for the determination of Coliform count was Eosin Methylene Blue (EMB) Agar. Cultural characteristics were read after 18-24 hours of incubation at 35 ^{0}C. Rapid lactose fermenters produced red colonies surrounded by red purple hallow. Slow lactose fermenters and late fermenters produced pale colonies. Counting was done by using a bacteriological colony counter and all those plates yielding >25 and <250 baterial colonies were taken into account.

3.12.7.iii) Test for Staphylococcal bacteria

The collection, preparation of samples, dilution and plating for the determination of staphylococcal count was similar to that of the above steps followed in the estimation of Total Plate Count. The plating media used for the determination of staphylococcal count in meat was Baird-Parker Agar. Cultural characteristics were observed after 24 to 48 hours of incubation at 35°C. Clear zones with grey black colonies in this medium were diagnostic for coagulase positive staphylococci. Counting was done by using a bacteriological colony counter and all those plates yielding >25 and < 250 bacterial colonies were taken into account.

3.12.7.iv) Test for *Salmonella typhimurium*

Test for the presence or absence of Salmonellae organisms in the chips samples were done by the method as described by Harrigan and McCance (1976). The samples were screened for salmonellae at 10 days interval of storage of period of 30 days.

Twenty-five grams meat sample was transferred aseptically to 250ml of nutrient broth and incubated at 37^{o}C for 24h. From this resuscitated culture, 10ml broth was transferred to 100ml tetrathionate broth and was incubated at 37^{o}C for 72h. A loop full of broth medium was streak inoculated on brilliant green agar (BGA). Typical colonies of salmonellae on BGA showed a pink or red (occasionally colourless) surrounded by a zone of bright red medium.

3.12.7. v) Test for presence of Yeast and Mould Count

Yeast and mould counts of the chips samples were made at similar time intervals as that of the total viable plate count by inoculating the appropriate dilution of the sample on Rose Bengal Chloramphenicol Agar Base, pH 7.2±0.1 and on incubating at 37^{o} C up to 48 hours (Harrigan and McCance, 1976). Counting was done by using a bacteriological colony counter and all those plates yielded >25 and <250 bacterial colonies were taken into account.

3.12.8. Sensory evaluation

Sensory evaluation of control and treatment spent hen meat chips were performed, utilizing an eight point hedonic score card (Keeton, 1983) with slight modifications, where 8=excellent and 1=extremely poor.

3.12.9. Cost of production:

The production cost of ready-to-cook chicken chips were determined by taking into account the basic price of deboned chicken meat, other non-meat ingredients namely binders, extenders, spices and condiments besides the cost of making. Production cost for all the treatment groups were estimated and compared separately to find out the relative production economy.

3.13. DATA ANALYSIS

The data obtained on studying different parameters were analyzed statistically as per the methods described by Snedecor and Cochran (1994) by employing the SAS, Version 2 software.

3.14. COLLABORATION WITH OTHER DEPARTMENTS

Department of Livestock Products Technology and AICRP on PHET, Department of Veterinary Biochemistry, Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022.

FIG. 3.1: FRESH FENUGREEK LEAVES

FIG. 3.2: DRIED FENUGREEK LEAVES

FIG. 3.3: POWEDERED FENUGREEK LEAVES

FIG. 3.4: FRESH FENUGREEK SEED

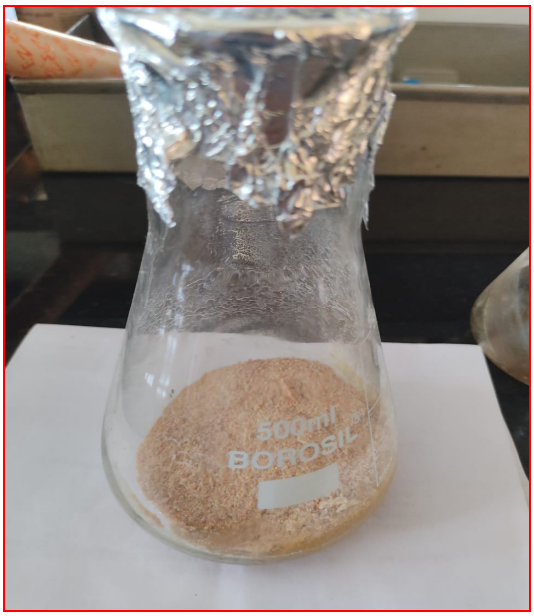


FIG. 3.5: POWDERED FENUGREEK SEEDS

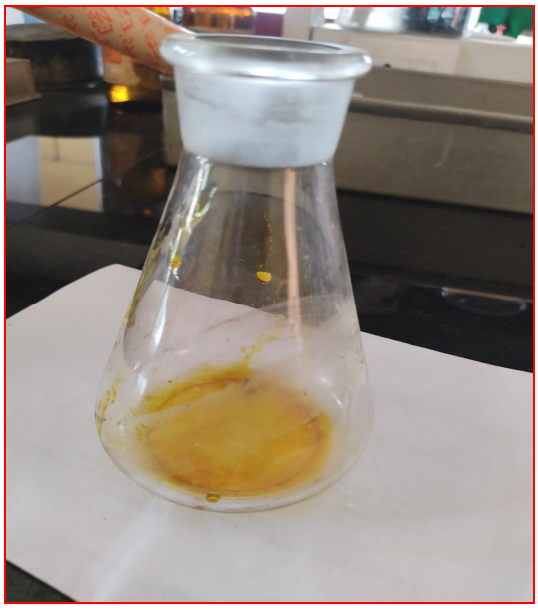


FIG. 3.6: EXTRACT OF FENUGREEK SEEDS

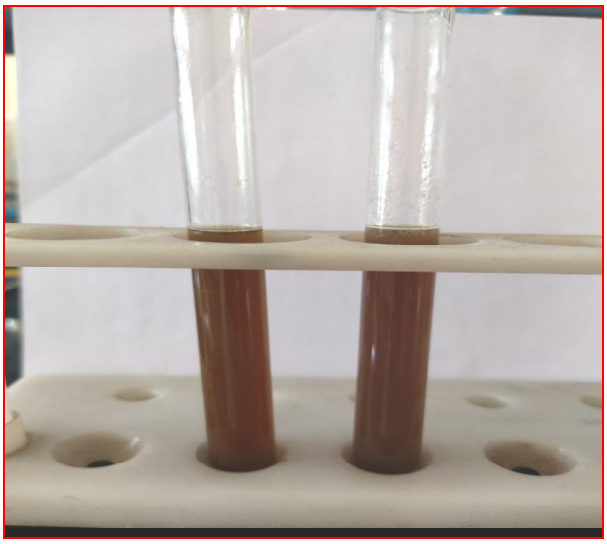


FIG. 3.7: SALWOKI TEST FOR STEROIDS WITH APPEARANCE OF REDDISH COLOUR

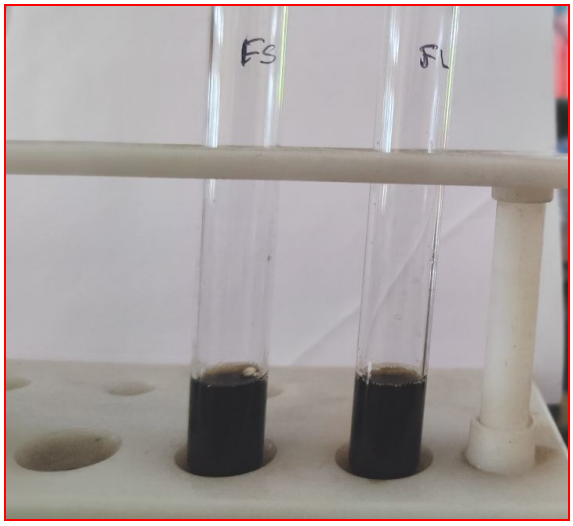


FIG. 3.8: TEST FOR PHENOLIC COMPOUNDS WITH APPEARANCE OF DARK BLUE COLOUR

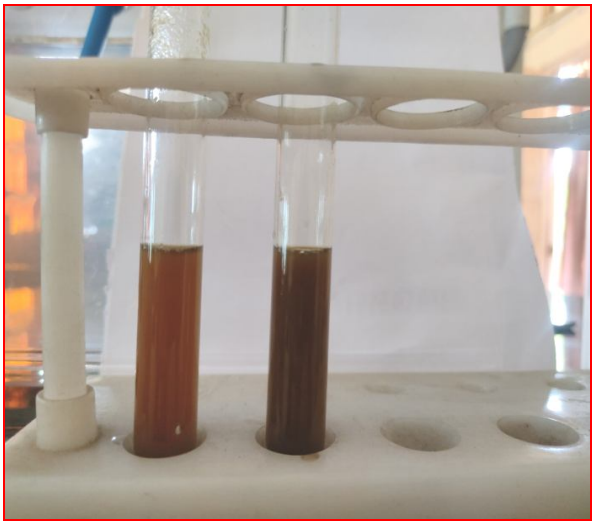


FIG. 3.9: TEST FOR TANNINS WITH APPEARANCE OF BROWNISH COLOUR

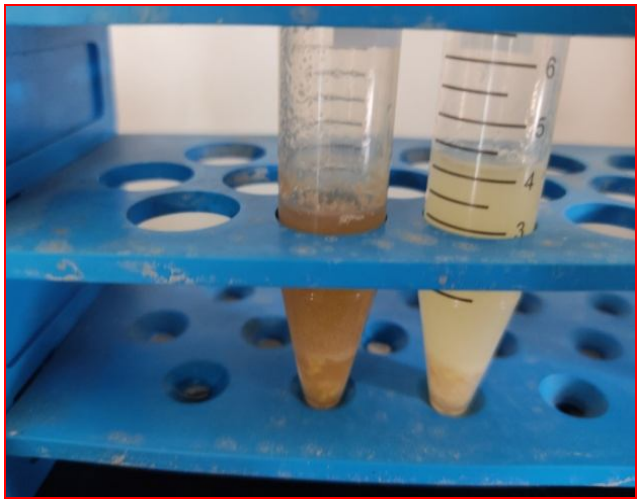


FIG. 3.10: TEST FOR SAPONIN WITH FORMATION OF PRECIPITATE

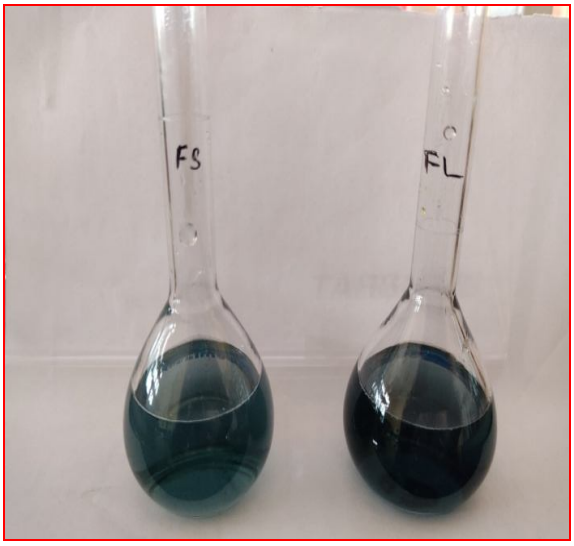


FIG. 3.11: TEST FOR FLAVANOIDS WITH APPEARANCE OF GREENCOLOUR

FIG. 3.12: TEST FOR ALKALOIDS WITH FORMATION OF PRECIPITATE

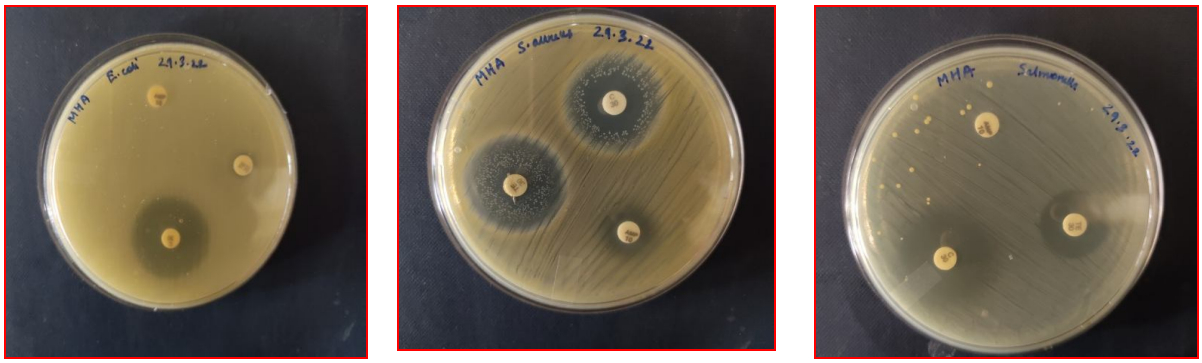


FIG. 3.13 (i, ii, iii): POSITIVE CONTROL

FIG. 3.13 (iv, v, vi): ANTIBACTERIAL ACTIVITY OF FENUGREEK LEAVES

FIG. 3.13 (vii, viii, ix): ANTIBACTERIAL ACTIVITY OF FENUGREEK SEEDS

FIG. 3.14: CONTROL

FIG. 3.15: TREATMENT A

FIG. 3.15: TREATMENT B

FIG. 3.15: TREATMENT C

CHAPTER - IV

Results and Discussion

DEVELOPMENT OF READY-TO-COOK CHICKEN CHIPS USING SPENT HEN MEAT INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

CHAPTER-IV

RESULTS AND DISCUSSION

The present work on the "Development of ready-to-cook chicken chips using spent hen meat incorporated with fenugreek seeds and/or leaves powder" was conducted using different levels of fenugreek leaves or seeds powder partially replacing poultry meat.

In the first phase of the research work, series of trials were carried out to standardize the ingredients and processing technologies for the development of the chicken chips using spent hen meat, refined wheat flour, rice flour, baking powder, salt, condiments and spices as non-meat ingredients. The formulation for chicken chips was standardized by series of sensory and physicochemical evaluations. The selected formulation was then incorporated with different levels of fenugreek leaves and fenugreek seeds replacing the spent hen meat at the rate of 0.00, 0.25, 0.50 and 1.00 per cent, each.

Samples of all the treatment groups were analyzed for various proximate and physicochemical properties as well as sensory evaluation.

4.1. PROXIMATE ANALYSIS OF FENUGREEK SEEDS AND LEAVES

The mean ± SE values for proximate parameters for fenugreek seeds and leaves are given in the Table 4.1 and graphically represented in Fig.4.1.

TABLE 4.1: MEAN PROXIMATE COMPOSITION OF FENUGREEK LEAVES AND SEEDS

PARAMETER (%)	FENUGREEK LEAVES	FENUGREEK SEEDS
Moisture	85.64 ± 0.72	10.26 ± 0.15
Crude Protein	4.62 ± 0.14	26.86 ± 0.10
Ether Extract	0.94 ± 0.01	10.72 ± 0.12
Crude Fibre	1.69 ± 0.13	47.52 ± 0.39
Total ash	10.73 ± 0.12	3.82 ± 0.07

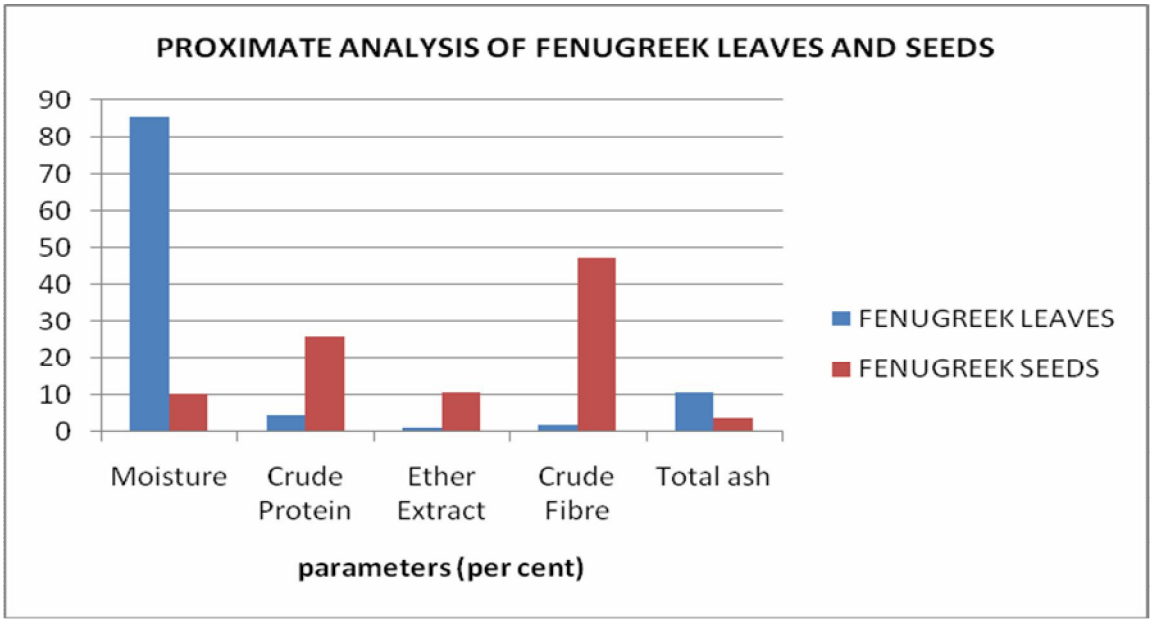


FIG. 4.1 GRAPHICAL REPRESENTATION OF PROXIMATE COMPOSITION OF FENUGREEK LEAVES AND SEEDS

It is a fact that the moisture content of seed or leaf is affected by the relative humidity of the surrounding environment as well as storage period. The present findings are in agreement with Gopalan *et al.* (1992) who found the moisture per cent of fenugreek leaves and fenugreek seeds to be 86.1% and 13.7%, respectively. Similar results on fenugreek seeds were also reported by Das and Lakkapa (2017) as 9.0%; Afzal *et al.* (2016) as 10.65%; Buba and Abdulrahman. (2015) as 10.91%; Krishan *et al.* (2013) as 10.42 to 11.51%; Snehlata and Payal (2012) as 12% and Naidu *et al.* (2011) as 10.78 to 11.53%.

Ether extract indicates the free fatty lipids of the sample. In the present work the values were found to be 0.94 ± 0.01% for leaves and 10.72 ± 0.12% for seeds. The result of this study was comparable with the findings of Singh *et al.* (2015b) and Naidu *et al.* (2011). Comparatively low fat content in fenugreek leaves might contribute for enhanced storage life due to reduced chance for lipid peroxidation. However, it might not be a good source of fat soluble vitamins nor supports as energy source.

Ash is the substance that remains after burning an organic matter. It contains almost all macro- as well as micro- nutrients except the organic carbon and nitrogen components (Singh *et al.,* 2013). The ash content in the present study was similar to the reports of previous workers (Mullaicharam *et al.,* 2013).

Fenugreek seed was reported to be rich in protein with a well balanced amino acid pattern (Feyzi *et al.,* 2014). The protein values obtained in the present study are similar to those reported by Tori (2011); Mullaicharam *et al.* (2013); and Isikili *et al.* (2005), wherein they reported proportion of protein ranging from 20 to 30%. These differences might be due to the variation in climatic condition, storage temperature, type of vegetation, rainfall or cultivation practice followed. However in other studies, Mathur and Choudhry (2009) and Naidu *et al.* (2011) reported protein values of fenugreek seed as 22% and 43.8%, respectively.

4.2. ANTIOXIDANT ACTIVITY ANALYSIS OF FENUGREEK LEAVES AND SEEDS

4.2.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The per cent inhibition of DPPH radical by using Fenugreek leaves or seeds are presented in Table 4.2. The reactivity of the extracts prepared from fenugreek leaves as well as seeds were analyzed using 2,2-diphenyl-1-picrylhydrazyl, a stable free radical which got reduced by accepting hydrogen or electron from the donor molecule. The mean per cent value of inhibition of DPPH radical by ethanolic extract at 50 µg/ml concentration were observed to be 40.04 ± 0.13 and 54.77 ± 0.14 for fenugreek leaves and fenugreek seed, respectively. The mean per cent inhibition of DPPH radical by ethanolic extract at 100 µg/ml concentration were observed to be 44.11 ± 0.09 and 60.96 ± 0.16 for fenugreek leaves and fenugreek seed, respectively. The mean per cent inhibition of DPPH radical by ethanolic extract at 150 µg/ml concentration were observed to be 56.42 ± 0.12 and 66.46 ± 0.11for fenugreek leaves and fenugreek seed, respectively. The mean per cent inhibition of DPPH radical by ethanolic extract at 200 µg/ml concentration were observed to be 65.03 ± 0.14 and 75.38 ± 0.08 for fenugreek leaves and fenugreek seed, respectively.

TABLE 4.2: THE MEAN ± PER CENT INIHIBITION OF DPPH RADICAL BY EXTRACTS OF FENUGREEK LEAVES AND FENUGREEK SEEDS

CONCENTRATION (µg/ml)	FENUGREEK LEAVES EXTRACT	FENUGREEK SEEDS EXTRACT	ASCORBIC ACID (STANDARD)
50	$40.04^{aA} \pm 0.13$	$54.77^{aB} \pm 0.14$	76.92
100	$44.11^{aA} \pm 0.09$	$60.96^{bB} \pm 0.16$	78.08
150	$56.42^{bA} \pm 0.12$	$66.46^{cB} \pm 0.11$	79.23
200	$65.03^{cA} \pm 0.14$	$75.38^{dB} \pm 0.08$	80.38^{b}
MEAN	$51.40^{A} \pm 2.27$	$64.39^{B} \pm 1.73$	78.65^{C}

Mean bearing different superscripts within a row (Lowercase) and within a column (Uppercase) differ significantly ($P<0.05$)

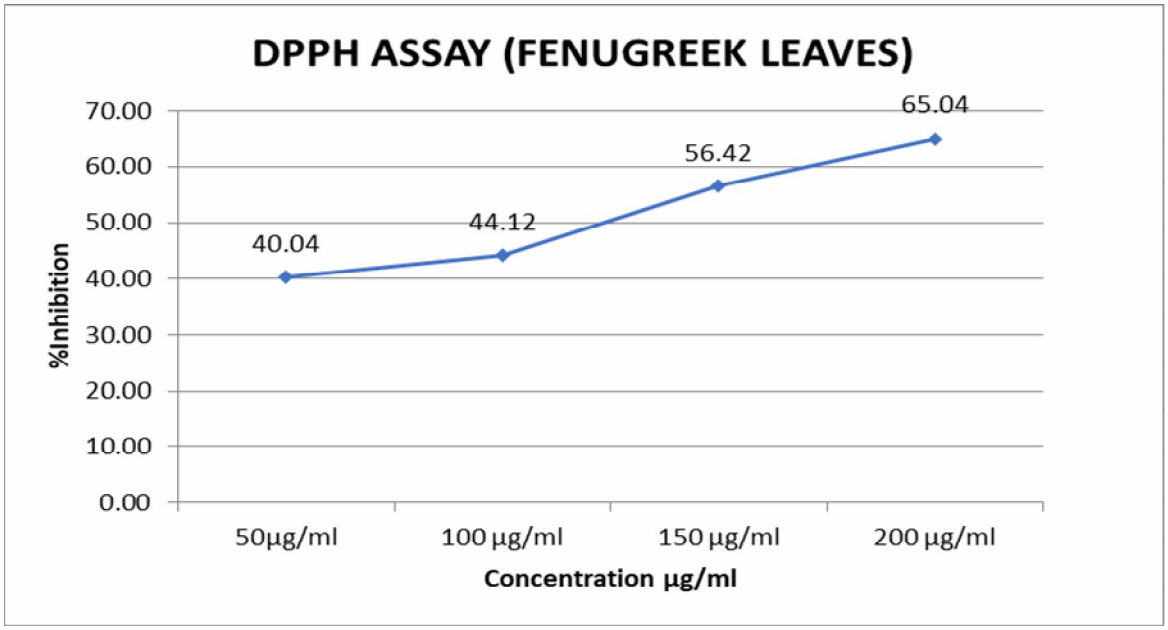


FIG. 4.2: INHIBITORY ACTIVITY OF FENUGREEK LEAVES AT DIFFERENT CONCENTRATION

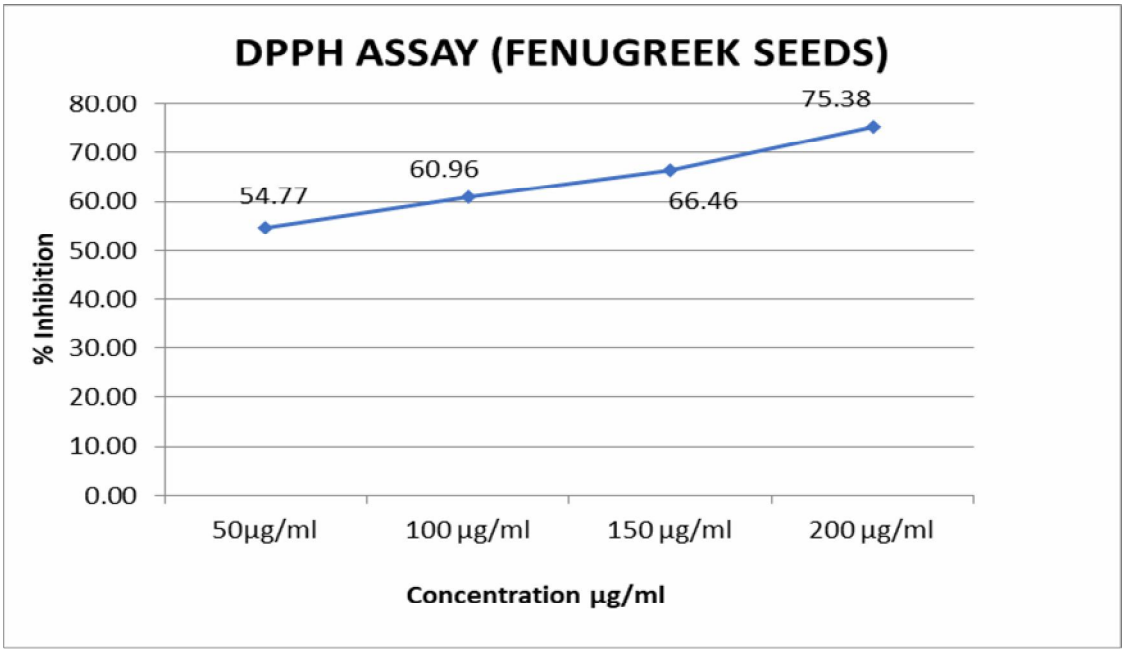


FIG 4.3: INHIBITORY ACTIVITY OF FENUGREEK SEEDS AT DIFFERENT CONCENTRATION

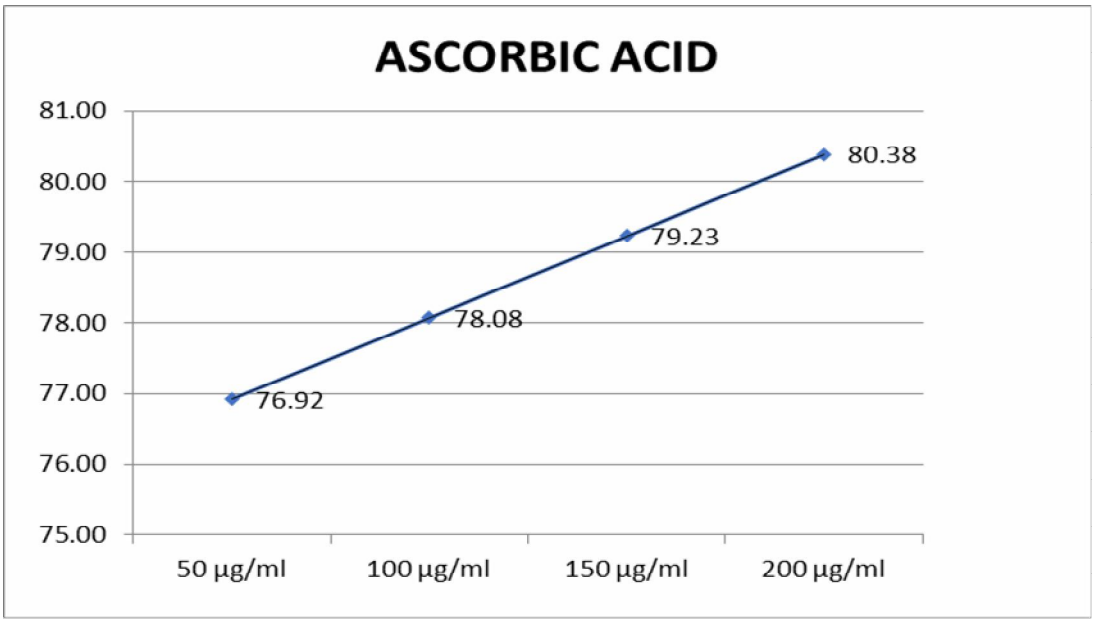


FIG 4.4: STANDARD GRAPH OF ASCORBIC ACID

The ethanolic extract derived from plant extracts were found to exhibit impressive antioxidant activity. Extracts derived from seeds of fenugreek showed the spectacular inhibition percentage against DPPH radical. There were significance differences ($P<0.05$) among all the extracts of fenugreek seeds, especially beyond the levels of 100 µg/ml. The present results are in agreement with the findings of Akbari *et al*. (2018), Yadav and Chowdhury (2017), Shinde *et al.* (2015) and Pathak *et al.* (2014) who studied the antioxidant activity against 2,2- diphenyl-1-picrylhydrazyl (DPPH) of fenugreek seed oil indicating a strong antioxidant radical scavenging activity against DPPH assays with an IC50 (Inhibitory Concentration) of 172.6 ± 3.1. The reactivity of ethanolic extract of fenugreek extract was analyzed with DPPH. As DPPH picked up one electron in the presence of a free radical scavenger, the absorption decreased and the resulting discolouration was related to the number of electrons gained (Silva *et al*, 2004). Among the two groups, ethanolic extract of fenugreek seeds showed the greater inhibition percentage than its leaf counterpart.

TABLE 4.3: ANOVA OF DPPH RADICAL INHIBITION BY EXTRACTS OF FENUGREEK LEAVES AND FENUGREEK SEEDS

Source of Variation	df	SS	MS	F
Treatment	1	337.5002	337.5002	60.26484**
Concentration	3	606.0745	202.0248	36.07404**
Error	3	16.80085	5.600284	

4.2.2. Total Phenolics content

The mean ± SE total phenolic content (TPC) for different concentrations of extracts of fenugreek leaves as well as fenugreek seeds showed significant ($P<0.05$) differences (Table 4.4). Also it was found to have significantly ($P<0.01$) higher concentrations (15.13 ± 0.02 mg GAE/g) in fenugreek seeds extracts than fenugreek leaves extract.

TABLE 4.4: THE MEAN (±SE) Of TOTAL PHENOLICS CONTENT (mg GAE/g) OF FENUGREEK LEAVES AND FENUGREEK SEEDS EXTRACTS

CONCENTRATION (µg/ml)	FENUGREEK LEAVES EXTRACT	FENUGREEK SEEDS EXTRACT	GALLIC ACID (STANDARD)
50	$4.04^{aA} \pm 0.13$	$14.98^{aB} \pm 0.06$	15.70
100	$4.71^{bA} \pm 0.14$	$15.16^{bB} \pm 0.04$	17.60
150	$4.99^{cA} \pm 0.07$	$15.17^{bB} \pm 0.03$	18.30
200	$5.09^{cA} \pm 0.09$	$15.20^{bcB} \pm 0.04$	19.10
MEAN	$5.16^{A} \pm 0.06$	$15.13^{B} \pm 0.02$	20.10

Mean bearing different superscripts within a row (Uppercase) and within a column (Lowercase) differ significantly (P<0.05)

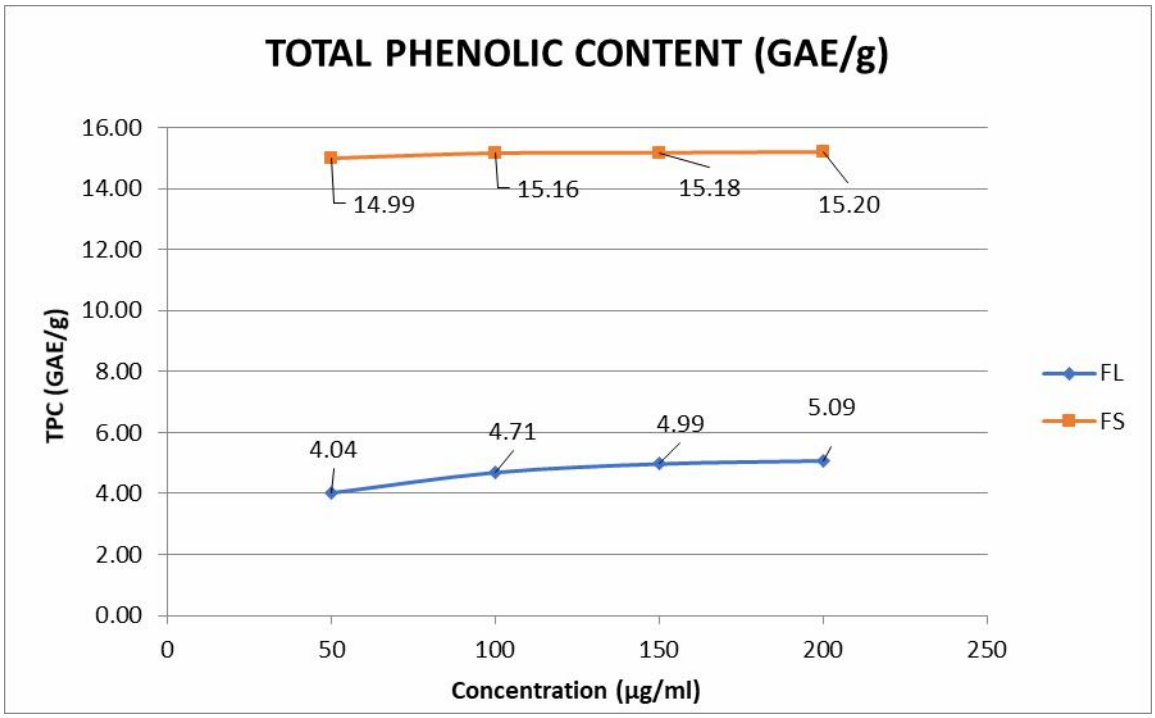


FIG 4.5: TOTAL PHENOLIC CONTENT OF FENUGREEK LEAVES AND SEEDS AT DIFFERENT CONCENTRATIONS

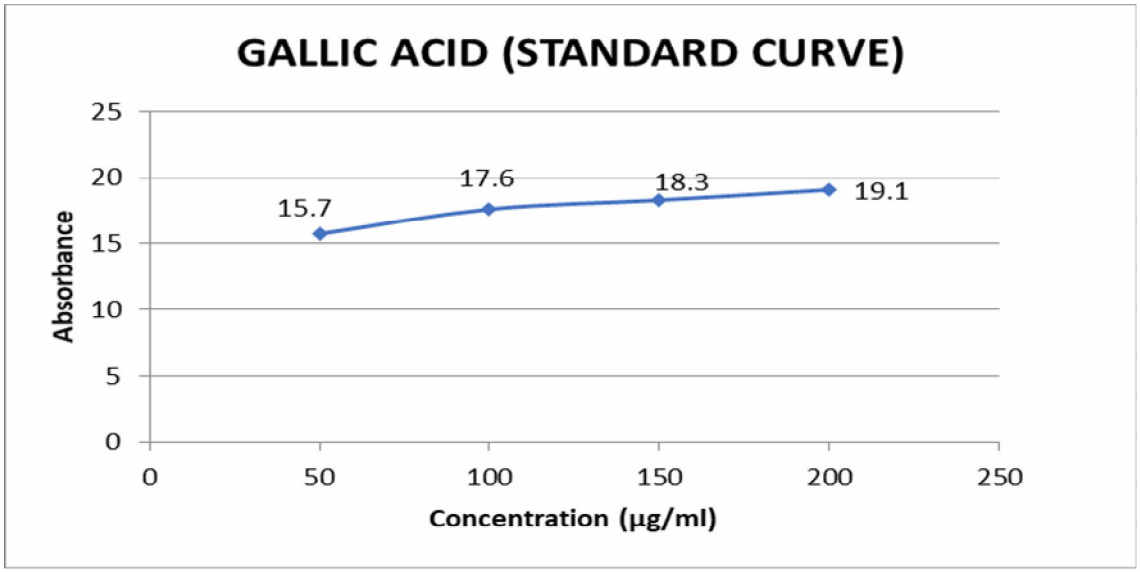


FIG 4.6: STANDARD GRAPH OF GALLIC ACID

In similar trials conducted by Seasotiya *et al.* (2014) and Bukhari *et al*. (2008), the total phenolic content was reported as 6.85mg GAE/g and 186mg GAE/g, respectively from methanolic seed extract. It could be seen that different solvent systems can be used to obtain better antioxidant activity as well as the extraction yield of that particular phytochemical. The variations between the results may be due to the differences in environmental conditions prevailed during the cultivation of the seed, sampling technique used etc. Concentrations and corresponding absorbance of Gallic Acid using Systronics Spectrophotometer 106 is shown in Table 4.4. The antioxidant activity of phenolic components was mostly because of its redox properties as they play major role as free radical scavengers, reducing agents, complexes of metals (Mathew *et al.*, 2015). In the present study, estimation of total phenolics has showed its antioxidant properties from the extraction of fenugreek leaves and seeds. The results are in agreement with the experiment conducted by Tsao and Deng (2004) where phenolics were used as alcoholic extract. Bukhari *et al*. (2008) in their trial work elaborated the findings of phenolic compounds present in fenugreek seeds.

TABLE 4.5: ANOVA OF TOTAL PHENOLICS (mg GAE/g) FOR DIFFERENT CONCENTRATIONS OF FENUGREEK LEAVES AND FENUGREEK SEED EXTRACTS

Source of Variation	df	SS	MS	F
Treatment	1	205.627	205.627	39543.12**
Concentration	3	0.127201	0.0424	8.153771*
Error	3	0.0156	0.0052	

4.2.3. Reducing ability

In this assay, the yellow colour of the solution changed to various shades of green and blue based on the reducing power of the component present in the extracts. This is due to the reduction of ferrycyanide complex by the reducing compound of plants.

TABLE 4.6: MEAN ±SE ABSORBANCE FOR REDUCING ABILITY (unit) OF DIFFERENT EXTRACTS OF FENUGREEK LEAVES AND FENUGREEK SEEDS

CONCENTRATION (µg/ml)	FENUGREEK LEAVES EXTRACT	FENUGREEK SEEDS EXTRACT	Butylated Hydroxy Toulene (STANDARD)
100	$0.25^{aA} \pm 0.02$	$0.47^{aB} \pm 0.06$	1.04
200	$0.27^{aA} \pm 0.02$	$0.53^{bB} \pm 0.02$	1.64
300	$0.35^{bA} \pm 0.19$	$0.71^{cB} \pm 0.04$	2.29
400	$0.51^{cA} \pm 0.05$	$0.89^{dB} \pm 0.05$	3.25
MEAN	$0.35^{A} \pm 0.03$	$0.65^{B} \pm 0.04$	2.05

Means bearing different superscripts within a row (Uppercase) and within a column (Lowercase) differ significantly ($P<0.05$)

The antioxidant can donate an electron to free radicals, which leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of ferrycyanide complex. Higher the absorbance value more is the reducing power of the extracts. The reducing activity of the extracts also increases with the increase in concentrations. Significant ($P<0.05$) differences in absorbance values of extracts of fenugreek seeds and leaves were observed. In the present study, increasing trend of reducing power was observed with increase in concentration of the extracts of both the fenugreek seed and leaves. Bukhari *et al.* (2008) revealed that the reducing power of the extracts of fenugreek seeds increased with an increase in the amount of the extract from 1.0 to 11.0%. The amount of the phenolic compounds was found in the ethanolic extract of fenugreek leaves and seeds. Therefore; similar results were obtained in reducing power activities. Hence, by correlating these results; it could be implied that there might be relationship between the amount of total phenolic content and reducing power of the herb.

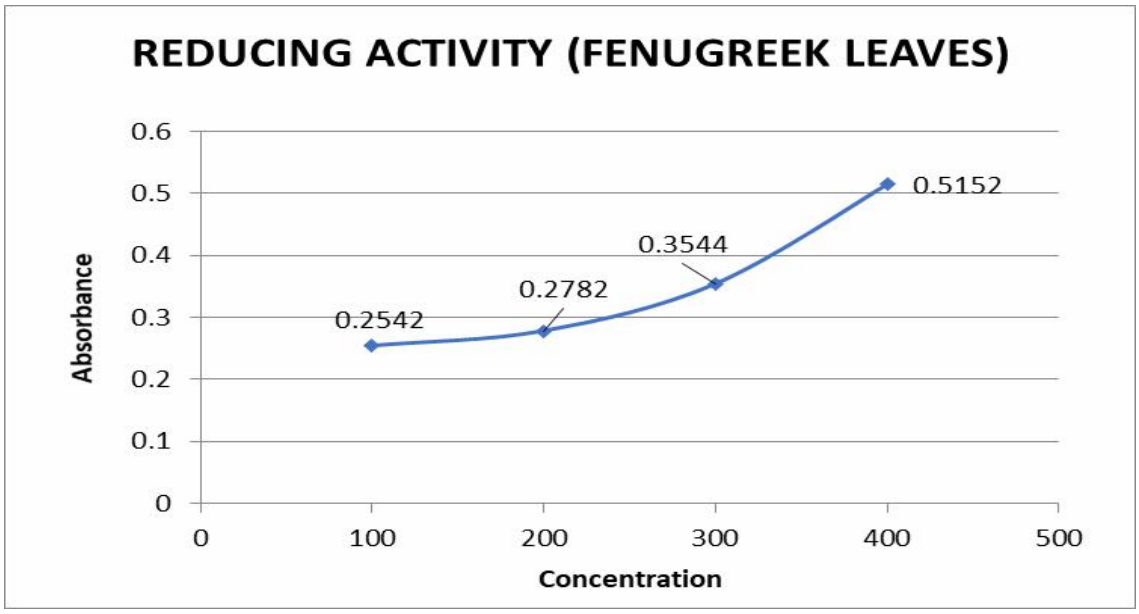


FIG 4.7: REDUCING ACTIVITY OF FENUGREEK LEAVES AT DIFFERENT CONCENTRATIONS

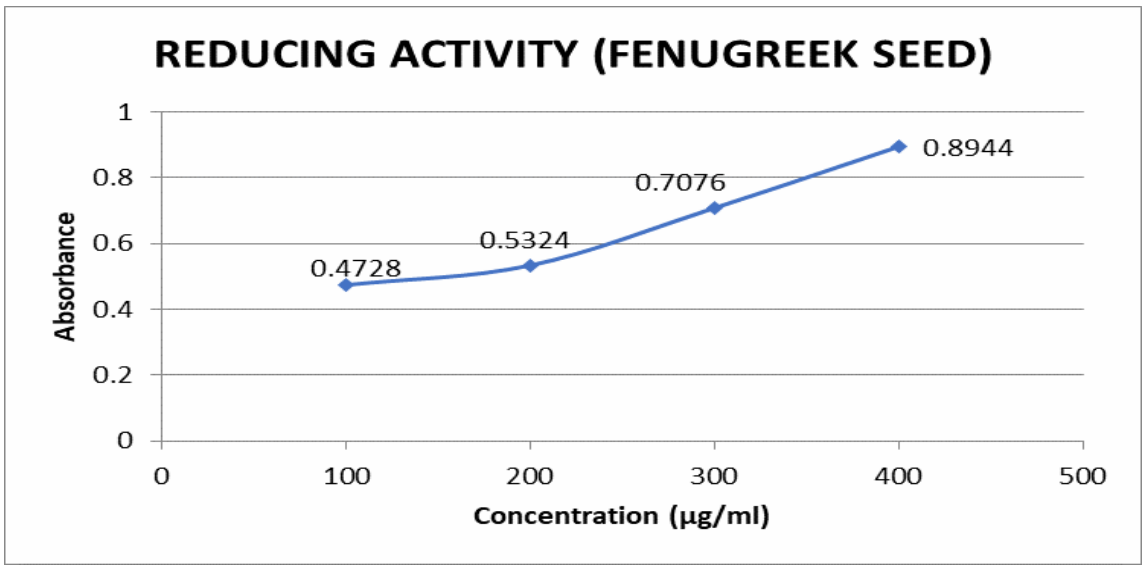


FIG 4.8: STANDARD CURVE FOR BHT

TABLE 4.7 : ANOVA ABSORBANCE FOR REDUCING ABILITY (Unit) OF DIFFERENT CONCENTRATIONS OF EXTRACTS OF FENUGREEK LEAVES AND FENUGREEK SEEDS

Source of Variation	df	SS	MS	F
Treatment	1	0.181563	0.181563	61.13341*
Concentration	3	0.140998	0.046999	15.82496*
Error	3	0.00891	0.00297	

4.3. QUALITATIVE PHYTO-CHEMICAL ANALYSIS OF FENUGREEK SEEDS AND LEAVES

Fenugreek leaves and seeds were analyzed for flavonoids, alkaloids, phenolics, tannins and steroids in ethanolic extracts. The present investigation confirmed that steroid, tannin, alkaloids, flavonoid, phenols and saponin were present in fenugreek leaves and seeds described in Table 4.8.

TABLE 4.8: PHYTOCHEMICAL SCREENING OF FENUGREEK LEAVES AND SEED

PHYTOCHEMICAL PARAMETERS	FENUGREEK LEAVES (n=5)	FENUGREEK SEEDS (n=5)
Steroid	+ve	+ve
Phenol	+ve	+ve
Tannin	+ve	+ve
Flavanoid	+ve	+ve
Alkaloid	+ve	+ve
Saponin	+ve	+ve

Earlier phytochemical studies indicated the presence of numerous valuable compounds, such as volatile compounds, flavonoids, phenolic compounds, steroids and alkaloids (Badgujar *et al.,* 2014). Tannins and saponins were found to be present in ethanol, methanol and aqueous extracts of fenugreek seeds (Chalghoumi *et al.,* 2020).

It is interesting to know that a fenugreek seed contain steroid saponins which increase the food consumption and induced hypocholesterolemia. Diosgenin, a naturally-occurring steroid saponin is found abundantly in fenugreek (Raju and Rao, 2012). Fenugreek contains approximately 4 to 8% saponins and about 1% alkaloids, which contributing to its bitterness (Mashkor, 2014). The phenolics identified in fenugreek are kaempfero-3-o-α-L-rhamnoside, kaempfero-3,7-o-α-L-diramnoside, quercetin 3,7-o- α-L-diramnoside and 3-o-α-L-rhamnosyl quercetin and these phenolics acts as antioxidants and help preventing lipid oxidation (Sauvaire *et al.,* 1996).

Trigonelline, a major alkaloid component found in fenugreek which has hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities, and it has been shown to reduce diabetic auditory neuropathy and platelet aggregation. It acts by affecting β cell regeneration, insulin secretion, activities of enzymes related to glucose metabolism, reactive oxygen species, axonal extension, and neuron excitability (Zhou *et al.,* 2012)

Three major flavonoids, tricin, naringenin and tricin-7-O-beta-D-glucopyranoside, are found in fenugreek. Flavonoids are putative antioxidant with high

activity of free radical scavenging and inhibit cyclooxygenase and lipoxygenase which are involved in initiation stage of inflammation reactions (Damas *et al.*, 1985).

4.4. ANTIBACTERIAL PROPERTIES OF FENUGREEK LEAVES AND SEEDS

The antibacterial potential of fenugreek leaves and seeds are presented in Table 4.9. The antibacterial activities were evaluated against Gram positive (*Staphylococcus aureus*) as well as Gram negative bacteria (*E coli, Salmonella typhymurium and Klebsiella spp.*) by well diffusion method. The antibacterial activities of both extracts (fenugreek leaves and seeds) exhibited positive reaction against *Staphylococcus aureus* and *Klebsiella spp.* at different concentrations. The diameter of the zone of inhibition was recorded in millimeter using a transparent scale. The zone of inhibition against *Staphylococcus aureus* ranged from 11 to 19 mm for fenugreek leaves and from 10-15 mm for fenugreek seeds. Likewise, the zone of inhibition against *Klebsiella spp.* ranged from 12 to 15 mm for fenugreek leaves and 13 to 16 mm for fenugreek seeds.

This study provides evidence that crude extracts of fenugreek seeds exhibited anti-bacterial effect against *E. coli* but no effect could be found with fenugreek leaves. Chalghoumi *et al*. (2016) also reported that fenugreek seeds crude extracts have antibacterial properties against *E. coli* depending on the solvent used for extraction. Alwhibi and Soliman (2014), in their experiment found that the chloroform and methanolic extracts of fenugreek seeds possessed significant antibacterial activity against *E. coli* ATCC25922. Whereas, Upadhyay *et al*. (2008) found that chloroform and acetone extracts of fenugreek seeds showed larger inhibition zone diameter compared to aqueous extract against *E. coli.*

In the present study no zone of inhibition could be observed for fenugreek leaves as well as fenugreek seeds against Salmonella spp. This could be attributed to the low concentration of extract used or improper diffusion of the extract. Hwa *et al.* (2019), Faraj *et al*. (2014), Marzougui *et al*. (2012), Massih *et al*. (2018) in their experiments found no results about **antimicrobial activity of fenugreek leaf or seed extracts under well diffusion method.**

The anti-bacterial potential is different depending on the concentration of the solvent utilized in the extraction process (Naz and Bano, 2012). Anti-bacterial substances present in the seed/leaf are dependent on the extracting solvent used. Quantitative phytochemical analyses to identify the compounds responsible for the anti-microbial activity also supports to the antibacterial properties.

The antimicrobial properties of the plant extract might be due to the presence of phenolic compounds in the extract. Phenol acted as an active compound which could destroy the cell membrane causing cell death (Hwa *et al.,* 2019).

TABLE 4.9: ZONE OF INHIBITORY ACTIVITY (IN MILLIMETERS) OF EXTRACTS OF FENUGREEK LEAVES AND SEEDS

ZONE OF INHIBITION (mm)						
Samples used in the wells	**Nature of samples**	**Concentration (µg/ml)**	***E coli***	***Salmonella***	***Staphylo-coccus***	***Klebsiella***
Fenugreek Leaves	Test sample	10	ND	ND	11	ND
	Test sample	50	ND	ND	19	12
	Test sample	100	ND	ND	13	15
Fenugreek Seeds	Test sample	10	ND	ND	10	15
	Test sample	50	8	ND	14	13
	Test sample	100	9	ND	15	16
Chloramphenicol	Positive control	30 mcg	22	16	20	18
Tetracycline	Positive control	30 mcg	14	14	22	20
Ampicillin	Positive control	10 mcg	16	18	20	14
Ethanol	Negative control	99.90%	-	-	-	-

ND - not detected

4.5. ANALYSIS OF THE TREATMENT GROUPS – PHASE I

4.5.1. PROXIMATE ANALYSIS

4.5.1. i) Moisture

The mean (±SE) values of moisture per cent in chicken chips under different treatment groups having fenugreek leaves and seeds are given in Table 4.10 and Table 4.12 and their analyses of variance in Table 4.11 and Table 4.13. The moisture levels of spent hen chips ranged from 4.68 ±0.04 to 4.98 ±0.06% values are reported to be in normal range. Some earlier workers reported the values within a range of 4.88 to 9.16% (Devalakshmi *et al.*, 2010), 6.59±0.04 to 10.32±0.04% (Singh *et al.*, 2013).

The moisture levels in all the treatment groups including the Control, progressively increased as storage period increased till 30th day of study. Under Control group, the values significantly increased from 0 day to 20th day beyond which there was no significant change. Under Treatment-I the moisture level did not increase till 10th day of storage. However, significant increase in moisture absorption recorded on 20th and 30th day of study. Similar trend was observed in the samples of Treatment II and III. However there was no significant difference among the four treatments including the Control.

Ockermann and Li (1999) found the moisture content in their dehydrated meat product, meat floss within the range of 3.47-5.23% while another study showed moisture content in Nigerian shredded chicken meat product (Danbunama) within the range of 4.22-4.50% (Ogunsola and Omojola, 2008). Chand *et al.*, (2013) prepared chicken meat based chips containing 60% spent hen meat and found to have moisture content less than 10%.

The increase in the moisture level may be due to the LDPE (Low Density Polyethylene) material used for packaging the product which aids in absorption of moisture from the surroundings. The findings are supported by the observations of Talukdar *et al.* (2015) wherein the moisture percentage of stored dried meat bhujia increased significantly ($P<0.05$) using LDPE aerobic packaging for 45 days at room temperature. The moisture from environment can be absorbed by the products packed with LDPE materials due to the less moisture-vapour barrier character of LDPE film

(Talukdar *et al*., 2015). Janardhana Rao (1997) also observed increased per cent moisture in chicken loaves due to the addition of 20% Bengal gram flour and 20% black grain flour. However, the present finding does not agree with the results of Singh *et. al*.(2011) who revealed a non-significant change in moisture per cent during storage period using aluminum foil/polyethylene. Sekhon and Bawa (1991) also noticed a significant decrease of moisture per cent in meat tikkas prepared from culled commercial hens and broiler breeder males packed in polyethylene bags (150 guage) under -18^{0}C for 5 months. Kausar *et al.* (2021) observed higher moisture contents ranged from 60.2 to 64.3% in nuggets prepared from goat meat with increasing level of fenugreek leaves (3.0, 6.0 and 9.0%).

TABLE 4.10: MEAN (±SE) VALUES OF MOISTURE (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	$4.68^{a} \pm 0.04$	$4.71^{a} \pm 0.06$	$4.72^{a} \pm 0.03$	$4.70^{a} \pm 0.03$
10	$4.74^{b} \pm 0.02$	$4.72^{a} \pm 0.03$	$4.73^{a} \pm 0.04$	$4.79^{a} \pm 0.02$
20	$4.87^{c} \pm 0.07$	$4.83^{b} \pm 0.08$	$4.84^{b} \pm 0.10$	$4.90^{b} \pm 0.05$
30	$4.88^{c} \pm 0.05$	$4.92^{c} \pm 0.09$	$4.93^{c} \pm 0.03$	$4.98^{c} \pm 0.06$

Means bearing different superscripts within a column differ significantly ($P<0.05$)
Ambient Temperature 37 ± 2^{0}C RH 80 ± 5%

TABLE 4.11: ANOVA FOR MOISTURE (%) VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.030684	0.010228	0.630988^{NS}
Days	3	0.663914	0.221305	13.65288*
Treatment X Days	9	0.026091	0.002899	0.178849^{NS}
Error	64	1.0374	0.016209	

TABLE 4.12 : MEAN (±SE) VALUES OF MOISTURE (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	$4.68^{a} \pm 0.04$	$4.66^{a} \pm 0.03$	$4.68^{a} \pm 0.01$	$4.67^{a} \pm 0.04$
10	$4.74^{b} \pm 0.02$	$4.78^{b} \pm 0.02$	$4.84^{b} \pm 0.03$	$4.84^{b} \pm 0.04$
20	$4.87^{b} \pm 0.06$	$4.88^{b} \pm 0.01$	$4.84^{b} \pm 0.04$	$4.82^{b} \pm 0.00$
30	$4.88^{b} \pm 0.04$	$5.02^{b} \pm 0.04$	$4.99^{c} \pm 0.01$	$4.99^{c} \pm 0.04$

Means bearing different superscripts within a column differ significantly (P<0.05)
Ambient Temperature 37 ± 2^{0} C RH 80 ±5%

TABLE 4.13: ANOVA FOR MOISTURE (%) VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of	df	SS	MS	F
Treatment	3	0.029	0.009	1.406^{NS}
Days	3	0.914	0.304	43.499*
Treatment X Days	9	0.082	0.009	1.300^{NS}
Error	64	0.448	0.007	

4.5.1. ii) Crude protein

The mean (±SE) values of crude protein per cent in chicken chips under different treatment groups having fenugreek leaves are given in Table 4.14 and their analyses of variance in Table 4.15. The crude proteins of chicken chips ranged from 22.33 ±0.22 to 22.44 ±0.08% values and are found to be within the desired range. According to the findings of other researchers, the crude protein values of chicken dried products are 48.72 ±1.03% (Muthulakshmi and Muthukumar, 2020), 41.07 to 42.97% (Kasthuri *et al.*, 2017) and 7.18 to 7.80% (Devalakshmi *et al*., 2010).

The analysis revealed no significant (P>0.05) differences among the various treatment groups as well as storage period.

TABLE 4.14: MEAN (±SE) VALUES OF CRUDE PROTEIN (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	22.34 ± 0.15	22.42 ± 0.27	22.41 ± 0.06	22.43 ± 0.18
10	22.33 ±0.22	22.42 ± 0.17	22.43 ± 0.07	22.42 ± 0.19
20	22.34 ±0.03	22.43 ± 0.04	22.42 ± 0.22	22.42 ± 0.06
30	22.34 ±0.26	22.44 ± 0.08	22.43 ± 0.13	22.42 ± 0.06

TABLE 4.15: ANOVA FOR CRUDE PROTEIN (%) VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.11013	0.03671	0.277453^{NS}
Days	3	0.0006	0.0002	0.001512^{NS}
Treatment X days	9	0.00171	0.00019	0.001436^{NS}
Error	64	8.46788	0.132311	

The mean (±SE) values of crude protein per cent in chicken chips under different treatment groups having fenugreek seeds are given in Table 4.16 and their analysis of variance in Table 4.17. The chicken chips incorporated with fenugreek seed powder found to have crude protein ranged from 22.75±0.07 to 22.85±0.09% compared to Control group 22.33±0.15%. Similar crude protein content was reported by Kumar *et al.* (2016) in meat biscuits ranging from 20.15 to 26.20%.

The protein percentage in the treatment groups (II and III) showed to have increased with increase in fenugreek seeds level (0.50 and 1.00%) compared to the Control and Treatment I (0.25%). However no significant changes were observed in crude protein in Treatment II and III.

The protein percentage significantly increased ($P<0.05$) in treatment products as compared to Control, which might be due to the contribution of vegetable protein from the fenugreek seeds (Hegazy, 2011).

Qureshi *et al.* (2018) also reported similar increasing trend in patties made of spent hen meat and using fenugreek seed powder at the range of 0.5 to 2.0%. Malav *et al.* (2017) prepared spent hen meat papad incorporated with corn and black gram flour and found significantly (P<0.05) higher protein (15.55±0.13 to 36.38%) content in all the treatment products under raw, microwave and fried stage than the Control.

Also, a significant (P<0.05) but gradual decrease in protein content was observed in beef burger during frozen storage up to a period of 3 months (Mahmoud *et al.,* 1987).

Kumar *et al*. (2016) found no significant difference in protein level of chicken meat biscuits when incorporated with wheat bran and oat bran at three different levels (3.0%, 5.0% and 7.0%) up to 180 days of LDPE storage at ambient temperature.

TABLE 4.16: MEAN (±SE) VALUES OF CRUDE PROTEIN (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	$22.34^{A} \pm 0.15$	$22.76^{B} \pm 0.14$	$22.85^{C} \pm 0.09$	$22.85^{C} \pm 0.09$
10	$22.33^{A} \pm 0.22$	$22.75^{B} \pm 0.20$	$22.84^{C} \pm 0.19$	$22.85^{C} \pm 0.23$
20	$22.34^{A} \pm 0.04$	$22.76^{B} \pm 0.09$	$22.84^{C} \pm 0.03$	$22.85^{C} \pm 0.05$
30	$22.34^{A} \pm 0.27$	$22.75^{B} \pm 0.07$	$22.85^{C} \pm 0.04$	$22.84^{C} \pm 0.09$

Means bearing different superscripts within a row differ significantly (P<0.05)

TABLE 4.17: ANOVA OF CRUDE PROTEIN (%) VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	3.495354	1.165118	10.77211*
Days	3	0.000304	0.000101	0.000936^{NS}
Treatment X Days	9	0.000551	6.13E-05	0.000566^{NS}
Error	64	6.92228	0.108161	

4.5.1.iii) Ether extract

The mean (±SE) values of ether extract per cent in chicken chips under different treatment groups having fenugreek leaves and seeds are given in Table 4.18 and 4.20 and their analyses of variance in Table 4.19 and 4.21. The chicken chips incorporated with fenugreek leaves powder and Control group presented to have ether extract per cent ranged from 4.81±0.06 to 4.88±0.03%. Data comparable to the present ether extract values are also reported by Sengar, (2014) who showed ether extract values in the range of 5.5 to 7.8%.

All the treatment groups and the Control did not show any significant difference up to the storage duration of 30 days under ambient condition. The data analysis revealed no significant (P>0.05) impact of storage on ether extract of chicken chips.

Contrary to the present findings, Qureshi *et al.* (2018) observed that fat content of spent hen meat patties added with 1.50% and 2.00% levels of fenugreek seed powder showed a significant (P<0.05) increase from Control group. Kausar *et al.* (2021), found the fat content of goat meat nuggets below 4% with addition of fenugreek leaves as functional additives at the rate of 3, 6 and 9%. The non-significant change observed in the present experiment might be due to the lower level of leaf/seed used in the study (0.5 to 1.0%).

TABLE 4.18: MEAN (±SE) VALUES OF ETHER EXTRACT (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	4.81 ± 0.06	4.82 ± 0.03	4.83 ± 0.02	4.83 ± 0.05
10	4.88 ± 0.03	4.84 ± 0.01	4.84 ±0.01	4.86 ± 0.01
20	4.86 ± 0.03	4.84 ± 0.05	4.85 ±0.07	4.88 ± 0.02
30	4.84 ± 0.02	4.85 ± 0.02	4.88 ±0.03	4.83 ± 0.02

TABLE 4.19: ANOVA FOR ETHER EXTRACT (%) VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.00117	0.00039	0.052274 ^{NS}
Days	3	0.01498	0.004993	0.669292^{NS}
Treatment X days	9	0.01409	0.001566	0.209842 ^{NS}
Error	64	0.47748	0.007461	

TABLE 4.20: MEAN (±SE) VALUES OF ETHER EXTRACT (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	4.81 ± 0.06	4.87 ± 0.02	4.90 ± 0.01	4.93 ± 0.02
10	4.88 ± 0.03	4.88 ±0.01	4.85 ± 0.01	4.86 ± 0.02
20	4.86 ± 0.03	4.86 ± 0.02	4.84 ± 0.02	4.87 ± 0.02
30	4.85 ± 0.02	4.86 ± 0.01	4.87 ± 0.03	4.86 ± 0.04

TABLE 4.21: ANOVA FOR ETHER EXTRACT (%) VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatments	3	0.008864	0.002955	0.708533^{NS}
Days	3	0.005624	0.001875	0.44954^{NS}
Treatment X days	9	0.034621	0.003847	0.922495^{NS}
Error	64	0.26688	0.00417	

4.5.1. iv) Total Ash per cent

The mean (±SE) values of total ash percentage in chicken chips under different treatment groups having fenugreek leaves are given in Table 4.22 and their analysis of variance in Table 4.23. The chicken chips incorporated with fenugreek leaves powder and Control group found to have total ash content ranged from 7.58±0.02 to 7.60±0.00%. Kasthuri *et al.* (2017) observed the ether extract values between 6.8 to 7.6% due to incorporation of drumstick leaf and jamun seed powder in chicken chips.

The analysis revealed no significant (P>0.05) differences among the various treatment groups.

In a research trial carried out by Kausar *et al.* (2021) prepared nuggets and found the ash content to increase with increasing level of goat meat and fenugreek leaf contents. Ash percentage of spent hen meat slices with blend of potato, texturized soy protein, whey protein concentrate, oat meal and barley flour was significantly higher than that of Control group (Gupta *et al.*, 2017) due to use of higher level of oats and barley flour which contributed to raise in total ash content of the product compared to low level of fenugreek powder (0.25- 1.0%) in the present study.

TABLE 4.22: MEAN (±SE) VALUES OF TOTAL ASH (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FL)	Treatment I (With 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	7.58 ± 0.02	7.58 ± 0.02	7.59 ± 0.02	7.58 ± 0.03
10	7.58 ± 0.03	7.59 ± 0.02	7.60 ± 0.01	7.60 ± 0.02
20	7.59 ± 0.02	7.60 ± 0.03	7.60 ± 0.02	7.59 ±0.02
30	7.60 ± 0.00	7.60 ± 0.01	7.60 ± 0.00	7.60 ± 0.03

TABLE 4.23: ANOVA FOR TOTAL ASH (%) VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.001255	0.000418	0.173537 ^{NS}
Days	3	0.003045	0.001015	0.421053 ^{NS}
Treatment x days	9	0.001215	0.000135	0.056002 ^{NS}
Error	64	0.15428	0.002411	

The mean (±SE) values of total ash percentage in chicken chips under different treatment groups having fenugreek seeds are given in Table 4.24 and their analysis of variance in Table 4.25. The analysis revealed significant (P<0.05) raise in total ash content among the three treatment groups. In a similar study conducted by Qureshi *et al.* (2018), the ash content improved only slightly by the addition of 1.5% fenugreek seed powder whereas, showed significant (P<0.05) increment at 2% incorporation level in meat patties. The increasing trend of ash content with higher level of fenugreeks powder might be due to the higher addition of fenugreek seed powder.

Contrary to the present findings Hegazy (2011) noted significantly decreased ash contents in the frozen stored chicken burger samples while using fenugreek seed flour.

TABLE 4.24: MEAN (±SE) VALUES OF TOTAL ASH (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	$7.58^{a} \pm 0.02$	$7.63^{ab} \pm 0.03$	$7.68^{bc} \pm 0.03$	$7.68^{c} \pm 0.08$
10	$7.58^{a} \pm 0.03$	$7.64^{ab} \pm 0.02$	$7.69^{bc} \pm 0.06$	$7.67^{c} \pm 0.07$
20	$7.61^{a} \pm 0.03$	$7.72^{b} \pm 0.08$	$7.68^{ab} \pm 0.05$	$7.70^{b} \pm 0.03$
30	$7.60^{a} \pm 0.00$	$7.72^{b} \pm 0.03$	$7.71^{b} \pm 0.05$	$7.71^{b} \pm 0.04$

Means bearing different superscripts within a row differ significantly (P<0.05)

TABLE 4.25: ANOVA VALUES FOR TOTAL ASH (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.156944	0.052315	4.932139*
Days	3	0.032054	0.010685	1.007326^{NS}
Treatment X Days	9	0.014731	0.001637	0.154316^{NS}
Error	64	0.67884	0.010607	

4.5.2. Physico-chemical analysis

4.5.2. i) pH

The mean (±SE) values of pH in chicken chips under different treatment groups having fenugreek leaves and seeds are given in Table 4.26 and 4.28 and their analyses of variance in Table 4.27 and 4.29.

In the present study, all the fenugreek leaves powder added treatment groups (Treatment I, II and III) along with the Control showed pH values ranging from 5.65 ± 0.01 to 5.68 ± 0.01 up to the duration of 20^{th} days of storage.

However, the fenugreek seeds powder added treatment groups (Treatment IV, V and VI) along with the Control showed pH values ranging from 5.61±0.01 to 5.68 ± 0.04 up to the duration of 20^{th} days of storage.

The analysis revealed significant ($P<0.05$) differences in pH with increase in storage period. No significant changes could be found between the different treatment groups and Control up to 20^{th} day of storage. However significant decrease in pH could be seen on 30^{th} day of storage in all the treatment groups incorporated with fenugreek leaves and/or seeds including that of Control.

TABLE 4.26: MEAN (±SE) VALUES OF pH IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	5.68^{a} ±0.02	5.67^{a} ± 0.01	5.66^{a} ± 0.02	5.67^{a} ± 0.04
10	5.66^{a} ± 0.02	5.68^{a}± 0.01	5.68^{a} ± 0.01	5.68^{a} ± 0.02
20	5.65^{ab} ± 0.01	5.66^{a} ± 0.01	5.67^{a} ± 0.03	5.67^{a} ± 0.01
30	5.62^{bc} ± 0.02	5.63^{b} ± 0.02	5.62^{b} ± 0.01	5.65^{b} ± 0.02

Means bearing different superscripts within a column differ significantly (P<0.05)

TABLE 4.27: ANOVA FOR pH VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.004004	0.001335	0.577117^{NS}
Days	3	0.023474	0.007825	3.383604*
Treatment X Days	9	0.004791	0.000532	0.23021^{NS}
Error	64	0.148	0.002313	

The decrease in pH during storage period might be attributed to the availability of more readily utilizable carbohydrate molecules and also due to the breakdown of muscle glycogen to lactic acid. The decrease in pH in meat products also depended on the presence of fermentable carbohydrates (Borch *et al.*, 1996). Singh *et al.* (2011) reported a decreasing trend in the pH of chicken snacks stored in laminated pouches under aerobic condition. Opposing to the current reports, increase in pH value in beef burgers during storage was repored by Hegazy, (2011). It was due to formation of some volatile nitrogen compounds, amines and hydrogen sulfide (Oroszvári, *et al.* 2005). In a study, Devalakshmi *et al.* (2010) reported the mean pH values of chicken meat chips to be increased significantly (P<0.01) as the storage period extended from 0 day to 8^{th} week in both ambient and refrigerated storage. Reddy and Rao (1996), Reddy and Vijayalakshmi (1996) found significant raise in the pH of raw and cooked chicken meat patties and sausages, respectively. This might be due to the fact that increasing pH during storage

can be related with the increase in water holding capacity of the meat. The reason for this increase is related to increase in solubility of meat proteins that move away from the isoelectric point (Karem, 2019).

There was non-significant change in pH values with dose related incorporation of fenugreek seeds in the chicken meat chips (Table 4.28). However, the pH values increased significantly with increase in the levels of fenugreek seed powder in spent hen meat incorporated patties (Qureshi *et al.,* 2018).

TABLE 4.28: MEAN (±SE) VALUES OF pH IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage interval (Days)	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	$5.67^{a} \pm 0.02$	$5.68^{a} \pm 0.02$	$5.68^{a} \pm 0.04$	$5.68^{a} \pm 0.01$
10	$5.66^{a} \pm 0.03$	$5.67^{a} \pm 0.02$	$5.68^{a} \pm 0.03$	$5.68^{a} \pm 0.04$
20	$5.65^{ab} \pm 0.01$	$5.68^{a} \pm 0.02$	$5.65^{ab} \pm 0.03$	$5.64^{b} \pm 0.01$
30	$5.61^{b} \pm 0.01$	$5.63^{b} \pm 0.00$	$5.65^{b} \pm 0.02$	$5.66^{ab} \pm 0.02$

TABLE 4.29: ANOVA OF VALUES FOR pH IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	SS	df	MS	F
Treatment	0.006664	3	0.002221	0.672597^{NS}
Days	0.019924	3	0.006641	2.010977^{*}
Treatment x days	0.005971	9	0.000663	0.2009^{NS}
Error	0.21136	64	0.003303	

4.5.2. ii) Tyrosine value

The mean (±SE) values of tyrosine in chicken chips under different treatment groups having fenugreek leaves and/or seeds are presented below (Table 4.30 and Table 4.32) and their analyses of variance in Table 4.31 and 4.33.

The tyrosine level ranged from 4.15 ± 0.03 to 4.19 ± 0.02. The values are comparable with the tyrosine of the chicken chips reported by Kasthuri *et al*. (2017) pepared using drumstick leaf and jamun seed powder.

The data analysis revealed no significant (P>0.05) differences among the various treatment groups or among the storage days.

TABLE 4.30: MEAN (±SE) VALUES OF TYROSINE IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	4.16 ± 0.07	4.17 ± 0.00	4.17 ± 0.03	4.16 ± 0.01
10	4.18 ± 0.02	4.16 ± 0.01	4.17 ± 0.01	4.18 ± 0.02
20	4.15 ± 0.03	4.17 ± 0.03	4.19 ± 0.02	4.16 ± 0.02
30	4.17 ± 0.01	4.17 ± 0.01	4.15 ± 0.01	4.17 ± 0.01

TABLE 4.31: ANOVA OF TYROSINE VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.000254	8.46E-05	0.024477 ^{NS}
Days	3	0.001124	0.000375	0.108398^{NS}
Treatment x days	9	0.006351	0.000706	0.204216 ^{NS}
Error	64	0.22116	0.003456	

These results contradict the work of Biswas *et al.* (2006) who recorded increase in tyrosine value with storage time. Tyrosine value is generally an indicator of proteolysis and has relationship with pH and exposure time that have lead to bacterial proteolysis. Tyrosine values tend to increase with increase in storage period due to deamination of amino acids which leads to the formation of amino acids (Pearson, 1968). The present study reveals no changes in tyrosine value which might be due to the characteristics dry condition of the product indicating the limitations of the bacterial growth.

TABLE 4.32: MEAN (±SE) VALUES OF TYROSINE IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	4.15 ± 0.07	4.19 ± 0.01	4.18 ± 0.05	4.21 ± 0.01
10	4.19± 0.01	4.20 ± 0.02	4.19 ± 0.01	4.20 ± 0.01
20	4.15 ± 0.03	4.16 ±0.02	4.18 ± 0.02	4.18 ±0.03
30	4.27 ± 0.02	4.24 ± 0.01	4.26 ± 0.02	4.24 ± 0.01

TABLE 4.33: ANOVA OF TYROSINE VALUES IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.00644	0.002147	0.598478 ^{NS}
Days	3	0.00679	0.002263	0.631004 ^{NS}
Treatment x days	9	0.00493	0.000548	0.152717 ^{NS}
Error	64	0.22956	0.003587	

4.5.2. iii). Water activity

The mean (±SE) values of water activity in chicken chips under different treatment groups having fenugreek leaves are given in Table 4.34 and their analysis of variance in Table 4.35.

In the present study the water activity of the product was found to be in the range of 0.67 ± 0.02 to 0.73± 0.00 which is quite low than the desired level for dried products (<0.99 in meat and meat products). The findings are comparable with the observations of Salgeuro *et al.* (1994) who recorded water activity from 0·60 to 0·91 in intermediate-moisture meat products stored under ambient condition (25^0C) for three months.

The analysis of variance revealed significant increase (P<0.05) in water activity in three treatment groups of chicken chips during the storage. The water activity of the groups was found to be reflected in lieu of with the moisture content of the products.

Malav *et al.* (2017) reported the water activity of the spent hen meat papad product to be significantly (P<0.05) higher during storage period of 45 days at room temperature of 25^0C. According to Lewicki (2004) and Rahman and Labuza (2007), changes in water content in the product resulted in changes in water activity, but both were not directly proportional.

Kumar *et al.* (2016) reported significant lower water activity in the chicken meat biscuits incorporated with wheat bran and oat bran.

TABLE 4.34: MEAN (±SE) VALUES OF WATER ACTIVITY IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	0.67^{A} ± 0.02	0.68^{A} ± 0.01	0.70^{A} ± 0.01	0.68^{A} ± 0.01
10	0.67 ^{A}± 0.01	0.69^{A} ± 0.01	0.69^{A} ± 0.01	0.69^{A} ± 0.03
20	0.67^{A} ± 0.00	0.69^{A} ±0.00	0.69^{A} ± 0.01	0.72^{B} ± 0.03
30	0.72^{B} ± 0.01	0.73^{B}± 0.00	0.72^{B} ± 0.00	0.72^{B} ± 0.01

Means bearing same superscripts within a column differ significantly (P<0.05)

TABLE 4.35: ANOVA OF VALUES FOR WATER ACTIVITY IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	***df***	***SS***	***MS***	***F***
Treatment	3	0.004676	0.001559	1.669628^{NS}
Days	3	0.017235	0.005745	6.154585*
Treatment x Days	9	0.005463	0.000607	0.650229^{NS}
Error	64	0.059742	0.000933	

The mean (±SE) values of water activity in chicken chips under different treatment groups incorporated with fenugreek seeds are given in Table 4.36 and their analysis of variance in Table 4.37. The data showed significant (P<0.05) increase in water activity of the treatment groups with prolong storage period. In Control, Treatment groups III and IV the water activity remained unchanged till the 20th day of storage, but increased significantly (P<0.05) thereafter. Whereas, in the Treatment group V, the water activity significantly increased (P<0.05) on 20th and 30th day of study.

TABLE 4.36: MEAN (±SE) VALUES OF WATER ACTIVITY IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	**Control (Without FS)**	**Treatment IV (with 0.25% FS)**	**Treatment V (With 0.50% FS)**	**Treatment VI (With 1.00% FS)**
0	0.67^{A} ±0.01	0.68^{A} ± 0.01	0.68^{A} ± 0.03	0.67^{A} ± 0.00
10	0.67 ^{A}±0.05	0.67^{A} ± 0.02	0.68^{A} ± 0.06	0.68^{A} ± 0.02
20	0.67^{A} ±0.02	0.67^{A} ± 0.02	0.68^{A} ± 0.01	0.71^{B} ± 0.03
30	0.72^{B} ±0.00	0.71^{B} ±0.02	0.72^{B} ±0.02	0.73^{B} ±0.01

Means bearing same superscripts within a column differ significantly (P>0.05)

TABLE 4.37: ANOVA OF VALUES FOR WATER ACTIVITY IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.00231	0.00077	0.273922^{NS}
Days	3	0.024701	0.008234	2.928797*
Treatment x Days	9	0.002842	0.000316	0.112341^{NS}
Error	64	0.179924	0.002811	

4.5.2. vi) Cooking yield

The mean (±SE) values of cooking yield in chicken chips under different treatment groups incorporated with fenugreek leaves are given in Table 4.38 and their analysis of variance in Table 4.39. The analysis revealed no significant (P>0.05) differences among the various treatment groups. Sen *et al.* (1994) observed that cooking yield of chicken loaves was not significantly affected after the replacement of minced meat by cooked and mashed potato at 0, 10, 15 and 20 % levels of formulations. Sharma and Rao (1996) also reported that there was a progressive but non-significant improvement in the cooking yield of chicken loaves with successively increasing levels (10 %, 15%, 20%) of pea flour.

Opposing to this trend significantly (P <0.01) increase mean cooking yield of chicken meat chips was noted in different formulations of chicken meat chips added with 15% Bengal gram flour and 15% black gram flour, separately by Devalakshmi *et al.* (2010).

Contrary to the present study, an increase in cooking yield of meat papad prepared with black gram and corn flour has been reported by Nagamallika and Rao (2010). Bhoyar *et al.* (1996) reported that cooking yields were significantly (P<0.01) more in the restructured chicken steaks added with texturized soya protein at 10, 20, and 30 per cent levels.

In a study conducted by Berwal *et al.* (1996), the cooking yields due to frying process were significantly (P<0.05) higher in comparison to papad prepared in microwave which might be due to uptake of oil. However, no cooking oil was used in the present study to attract the change in the product in terms of cooking yield.

TABLE 4.38: MEAN (±SE) VALUES OF COOKING YIELD (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	95.00 ± 1.77	91.33 ± 1.46	92.67 ± 1.58	91.55 ± 1.00
10	93.92 ± 0.88	91.29 ± 0.96	94.20 ± 1.15	91.40 ± 0.37
20	91.51 ± 0.77	92.09 ± 1.55	92.00 ± 0.68	92.17 ± 1.15
30	92.51 ± 0.88	92.54 ± 0.73	90.97 ± 0.76	91.64 ± 0.37

TABLE 4.39: ANOVA OF VALUES FOR COOKING YEILD (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	30.16274	10.05425	1.705502^{NS}
Days	3	11.03135	3.677115	0.623749^{NS}
Treatment x days	9	59.13911	6.571013	1.114641^{NS}
Error	64	377.2916	5.895181	

The mean (±SE) values of cooking yield in chicken chips under different treatment groups having fenugreek seeds are given in Table 4.40 and their analysis of variance in Table 4.41. The analysis of variance revealed no significant (P>0.05) differences among the various treatment groups.

TABLE 4.40: MEAN (±SE) VALUES OF COOKING YIELD (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	93.59 ± 0.18	91.88 ± 0.95	92.65 ± 0.98	92.80 ± 0.58
10	92.98 ±0.58	91.24 ± 1.42	93.15 ± 1.48	92.26 ±0.86
20	92.14 ±0.82	92.01 ± 0.38	92.53 ±0.65	91.83 ±0.68
30	92.48 ±0.43	91.16 ±0.78	91.33 ± 0.44	91.18 ±0.91

TABLE 4.41: ANOVA OF VALUES FOR COOKING YEILD (%) IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	16.60319	5.534395	1.591987 ^{NS}
Days	3	15.35929	5.119762	1.472716 ^{NS}
Treatment x Days	9	9.455365	1.050596	0.302207 ^{NS}
Error	64	222.49	3.476407	

Hegazy (2011) reported that the cooking yield per cent of beef burger samples containing fenugreek seed flour at levels of 9 and 12% was higher (P<0.05) than that of the Control. It has been reported that the products prepared from spent hen had high cooking loss due to high fat content and poor water binding capacity (Acton and Dick, 1978 and Buyck *et al.*, 1982). The non significant change in the present study might be due to the lower incorporation of fenugreek seed (0.25, 0.50 and 1.00 %) in the product.

4.5.3. Sensory evaluation for organoleptic quality

The organoleptic quality for traits like colour, flavor, texture, crispiness, after-taste and overall acceptability scores of the chicken meat chips under aerobic condition are given.

4.5.3. i) Colour scores

The freshly prepared chicken chips with addition of fenugreek leaves and/or seeds on day 1 exhibited good colour scores (5.3 to 5.6 out of 7 hedonic scale) for all the chips under different treatment groups. The scores obtained fall under ‘good’ category when compared to the hedonic score card thereby indicating a ‘good’ aesthetic appearance of the product. The overall mean colour scores of chicken meat chips as influenced by different fenugreek levels and at different storage periods are given in Tables 4.42 & 4.44 and the analysis of variance are presented in Tables 4.43 and 4.45.

The freshly prepared chicken chips exhibited good scores in all the treatment groups along with the Control group. The colour scores among all the treatment groups were comparable, which remained unchanged throughout the storage period of 30 days.

Falling in the line of the present findings, Hegazy (2011) found no significant differences among beef burger samples in terms of color scores up to 3 months of storage.

Table 4.42: COLOUR SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	5.5 ± 0.22	5.4 ± 0.26	5.4 ± 0.30	5.5 ± 0.30
10	5.6 ± 0.22	5.4 ± 0.22	5.4 ± 0.30	5.4 ± 0.26
20	5.6 ± 0.26	5.5 ± 0.26	5.6 ± 0.30	5.5 ± 0.34
30	5.6 ± 0.30	5.6 ± 0.30	5.3 ± 0.29	5.5 ± 0.26

TABLE 4.43: ANOVA OF SCORES FOR COLOUR IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Sample	3	0.275	0.091667	0.115183^{NS}
Columns	3	0.475	0.158333	0.198953^{NS}
Interaction	9	0.625	0.069444	0.08726^{NS}
Within	144	114.60	0.795833	

The present findings are supported by Mishra *et al*. (2015) who reported non-significant difference (P >0.05) in the treatments both in dried and rehydrated and cooked forms of chicken meat rings. Similar findings were also noted by Kasthuri *et al.* (2017) with no significant changes in colour scores in chicken chips incorporated with drumstick leaf and jamun seed powder.

Chicken chips containing 4% Flaxseed powder and 6% oats powder had scores of 6.37 and 6.47 out of 7 hedonic scale for appearance in the trial conducted by Kasthuri *et al.* (2018).

However, contrary to the findings of the present study, prolong storage period reflected significant (P< 0.01)) lower values in the mean colour scores of chicken chips compared to on 0^{th} day stored up to 7 weeks as reported by Devalakshmi *et al*.(2010). Inferior values were observed irrespective of the type of storage technique used either in aerobic (37^0C) or in refrigerated (7^0C) storage.

TABLE 4.44: COLOUR SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	5.70 ± 0.26	5.60 ± 0.34	5.60 ± 0.31	5.70 ± 0.33
10	5.50 ± 0.22	5.50 ± 0.31	5.60 ± 0.34	5.50 ± 0.31
20	5.50 ± 0.22	5.60 ± 0.30	5.60 ± 0.33	5.50 ± 0.31
30	5.70 ± 0.26	5.60 ± 0.22	5.50 ± 0.27	5.60 ± 0.30

TABLE 4.45: ANOVA OF SCORES FOR COLOUR IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	0.01875	3	0.00625	0.007252 ^{NS}
Days	0.36875	3	0.122917	0.142627^{NS}
Treatment x days	0.45625	9	0.050694	0.058824 ^{NS}
Error	124.1	144	0.861806	

4.5.3. ii) Texture scores

The mean texture scores of chicken meat chips as influenced by different fenugreek leaves and/or seeds levels and at different storage periods are given in Tables 4.46 & 4.48 and the analysis of variance are presented in Tables 4.47 and 4.49.

The texture scores for the fenugreek leaves treated chicken chips found to have no significant deviation from the Control group. The texture scores obtained by chicken

chips incorporated with fenugreek seeds and leaves powder showed a good score (5.30 ± 0.30 to 5.60 ± 0.31 out of 7 hedonic scale). Fenugreek has a property of changing the textural characteristic of the food due to presence of soluble fibre but in the present study the difference was not evident may be due to the low percentage of incorporation (0.25-1.0%). Notwithstanding the packaging material used, the reduction in texture score was found to be directly proportionate with the increase in the storage period up to 30 days under aerobic condition, however with no significant change. The packaging material was not found to have pronounced affect on the texture quality.

TABLE 4.46: TEXTURE SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	5.40 ± 0.26	5.60 ± 0.22	5.40 ± 0.27	5.50 ± 0.27
10	5.60 ± 0.22	5.40 ± 0.22	5.40 ± 0.31	5.40 ±0.27
20	5.60 ± 0.27	5.50 ± 0.27	5.60 ± 0.31	5.50 ± 0.34
30	5.60 ± 0.31	5.60 ± 0.31	5.50 ± 0.30	5.50 ± 0.27

Mishra *et al*. (2015) also did not find significant (P>0.05) different scores in texture while working with the product dehydrated chicken meat rings during storage under frozen at −20 °C temperature.

TABLE 4.47: ANOVA OF SCORES FOR TEXTURE IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	0.36875	0.122917	0.160181 ^{NS}
Days	3	0.21875	0.072917	0.095023^{NS}
Treatment x Days	9	0.90625	0.100694	0.131222 ^{NS}
Error	144	110.50	0.767361	

TABLE 4.48: TEXTURE SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	4.80 ± 0.32	4.90 ± 0.23	5.00 ± 0.33	4.90 ± 0.28
10	5.00 ± 0.29	5.10 ± 0.23	5.00 ± 0.21	4.90 ± 0.18
20	5.00 ± 0.29	4.90 ± 0.38	5.10 ± 0.35	4.90 ± 0.38
30	4.90 ± 0.35	5.10 ± 0.35	4.90 ± 0.34	5.00 ± 0.29

TABLE 4.49: ANOVA OF SCORES FOR TEXTURE IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	0.225	0.075	0.079063^{NS}
Days	3	0.225	0.075	0.079063^{NS}
Treatment x days	9	0.725	0.080556	0.084919^{NS}
Error	144	136.6	0.948611	

4.5.3. iii) Crispiness

Crispiness is an important sensory parameter for dried products like chips. The mean crispiness scores of chicken meat chips as influenced by different fenugreek leaves and seeds levels at different storage periods are given in Tables 4.50 & 4.52 and the analysis of variance are presented in Tables 4.51 and 4.53.

In the present study, good scores for crispiness as obtained in all the treatment groups including the Control group (5.8 ± 0.25 to 6.2 ± 0.36 out of 7 point hedonic scale). The product fall under good to very good category as judged by the panelists. The crispiness scores for chicken chips remained unchanged regardless of variation of treatment or length of storage.

Devalakshmi *et al.* (2010) also noticed a gradual but non-significant decline in the crispiness scores of chicken meat chips with prolong storage period of 8 weeks.

TABLE 4.50: CRISPINESS SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	6.0 ± 0.21	6.1 ± 0.18	5.9 ± 0.28	6.2 ± 0.20
10	6.1 ± 0.23	6.0 ± 0.26	6.2 ± 0.36	5.9 ± 0.38
20	5.9 ± 0.35	5.8 ± 0.25	5.9 ± 0.31	5.8 ± 0.36
30	5.9 ± 0.28	5.9 ± 0.23	5.8 ± 0.33	5.8 ± 0.29

TABLE 4.51: ANOVA OF SCORES FOR CRISPINESS IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	0.05	0.016667	0.020202^{NS}
Days	3	1.60	0.53333	0.646465^{NS}
Treatment x Days	9	1.15	0.127778	0.154882^{NS}
Error	144	118.8	0.0825	

TABLE 4.52: CRISPINESS SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	5.60 ± 0.22	5.60 ± 0.34	5.50 ± 0.37	5.60 ± 0.34
10	5.50 ± 0.43	5.60± 0.27	5.40 ± 0.22	5.40 ± 0.22
20	5.30 ± 0.30	5.30 ± 0.40	5.30 ± 0.37	5.20 ± 0.36
30	5.20 ± 0.36	5.00 ± 0.37	5.10 ± 0.35	5.10 ± 0.34

TABLE 4.53: ANOVA OF SCORES FOR CRISPINESS IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	0.16875	0.05625	0.050404^{NS}
Days	3	5.36875	1.789583	1.603609^{NS}
Treatment x Days	9	0.45625	0.050694	0.045426^{NS}
Error	144	160.7	1.115972	

4.5.3. iv) Flavor

The mean (±SE) values of flavor of chicken chips under different treatment groups having fenugreek leaves and seeds are given in Table 4.54 and 4.56 and their analyses of variance in Table 4.55 and 4.57.

From the data obtained on sensory evaluation of the chicken chip treated with fenugreek seeds and leaves powder showed low scores (2.10 ± 0.35 to 2.60 ± 0.30 out of 7 hedonic scale) in the Treatment II and Treatment III groups throughout the storage period up to 30 days. According to the taste perceptions of the panelists, the product evaluated as bitter due to addition of fenugreek leaves and seeds. However, Treatment group I with 0.25% level fenugreek leaves showed a better score (4.4 ± 0.22 to 4.9 ± 0.46out of 7 hedonic scale) compared to the previous Treatment groups.

The analysis of variance indicated significantly ($P<0.05$) lower scores with increase in level of fenugreek in the product.

TABLE 4.54: FLAVOUR SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	5.4^{A}± 0.31	4.6^{B} ± 0.31	2.9^{C} ± 0.28	2.6^{D} ± 0.37
10	5.1^{A} ± 0.18	4.4^{B} ± 0.22	2.9^{C} ± 0.38	2.5^{D} ± 0.40
20	5.4^{A} ± 0.27	4.4^{B} ± 0.40	2.9^{C} ± 0.23	2.2^{D} ± 0.25
30	5.5^{A} ± 0.27	4.9^{B} ± 0.46	3.0^{C} ± 0.39	2.3^{D} ± 0.37

Means bearing same superscripts within a row differ significantly ($P<0.01$)

TABLE 4.55: ANOVA OF SCORES FOR FLAVOUR IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	229.125	76.375	71.60156**
Days	3	1.275	0.425	0.398437^{NS}
Treatment x Days	9	2.375	0.263889	0.247396^{NS}
Error	144	153.60	1.066667	

TABLE 4.56: FLAVOUR SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	$6.10^{A} \pm 0.31$	$4.80^{B} \pm 0.38$	$2.40^{C} \pm 0.31$	$2.10^{C} \pm 0.35$
10	$5.70^{A} \pm 0.33$	$3.90^{B} \pm 0.52$	$2.60^{C} \pm 0.49$	$2.10^{C} \pm 0.41$
20	$5.80^{A} \pm 0.36$	$4.10^{B} \pm 0.35$	$2.60^{C} \pm 0.30$	$2.20^{C} \pm 0.29$
30	$5.80^{A} \pm 0.25$	$4.30^{B} \pm 0.37$	$2.30^{C} \pm 0.30$	$2.20^{C} \pm 0.25$

Means bearing same superscripts within a row differ significantly ($P<0.01$)

TABLE 4.57: ANOVA OF SCORES FOR FLAVOUR IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	354.225	118.075	92.40652**
Days	3	1.625	0.541667	0.423913^{NS}
Treatment x days	9	4.525	0.502778	0.393478^{NS}
Error	144	184	1.277778	

4.5.3. v) After taste

The mean (±SE) values of after-taste of chicken chips under different treatment groups having fenugreek leaves are given in Table 4.58 and their analyses of variance in Table 4.59. During sensory evaluation, the panelists scored the Control and Treatment I with better scores (5.00 ± 0.33 to 5.50 ± 0.48) than the Treatment II (2.80± 0.42 to 3.90 ± 0.53) and Treatment III (2.00 ± 0.33 to 2.80 ± 0.36) with increase in the level of addition of fenugreek leaves powder in the chicken chips (0.25, 0.50 and 1.0%). From the data, it can be seen that the chicken chips without addition of fenugreek and Treatment I with 0.25% fenugreek leaves showed no changes. However increasing the level of fenugreek leaves powder reduced the score for after-taste from 5.00 ± 0.37 to 2.80 ± 0.36 out of 7 scale of hedonic score card.

The formulations of chicken meat chips added with fenugreek leaves and seed powder had significantly ($P<0.01$) lower mean after-taste scores than the Control. The mean (±SE) scores for after-taste of chicken chips under different treatment groups having fenugreek seeds are given in Table 4.60 and their analysis of variance in Table 4.61. The analysis of variance revealed significant ($P<0.05$) differences among the various treatment groups. With increasing the level of fenugreek seeds powder reduced the score for after-taste from 5.70 ± 0.21 to 2.10 ± 0.31out of 7 scale of hedonic score card.

Supporting to current findings, Devalakshmi *et al.* (2010) also found a gradual decline in taste of chicken meat chips at both ambient (37+2°C) and refrigerated (7+1°C) storage stored up to 8 weeks.

The after-taste scores were significantly ($P<0.05$) higher in treatment products of meat papad using spent hen meat powder and corn flour black gram flour and combination of corn and black gram flour than the Control (Malav *et al.*, 2017).

Dissimilar results showed that there were no significant differences among beef burger samples with fenugreek seed flour in terms of taste (Hegazy, 2011).

TABLE 4.58: AFTER TASTE SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (With 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	$5.00^{A} \pm 0.37$	$5.00^{A} \pm 0.33$	$3.30^{A} \pm 0.50$	$2.80^{A} \pm 0.36$
10	$5.10^{A} \pm 0.18$	$5.20^{A} \pm 0.36$	$3.50^{A} \pm 0.40$	$2.50^{A} \pm 0.50$
20	$5.50^{AB} \pm 0.31$	$5.40^{AB} \pm 0.40$	$3.90^{AB} \pm 0.53$	$2.20^{AB} \pm 0.25$
30	$5.30^{AB} \pm 0.30$	$5.50^{AB} \pm 0.48$	$2.80^{AB} \pm 0.42$	$2.00^{AB} \pm 0.33$

Means bearing same superscripts within a row differ significantly (P<0.01)

TABLE 4.59: ANOVA OF SCORES FOR AFTER TASTE IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	11.525	3.841667	2.575419**
Days	3	262.675	87.89167	58.92179^{NS}
Treatment X Days	9	7.375	0.819444	0.549348^{NS}
Error	144	214.80	1.491667	

TABLE 4.60: AFTER TASTE SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FS)	Treatment IV (with 0.25% FS)	Treatment V (With 0.50% FS)	Treatment VI (With 1.00% FS)
0	$6.10^{A} \pm 0.18$	$5.00^{B} \pm 0.39$	$3.50^{C} \pm 0.49$	$2.30^{D} \pm 0.49$
10	$5.80^{A} \pm 0.33$	$3.60^{B} \pm 0.43$	$2.60^{C} \pm 0.31$	$2.10^{D} \pm 0.31$
20	$6.00^{A} \pm 0.36$	$4.10^{B} \pm 0.35$	$2.50^{C} \pm 0.34$	$2.20^{D} \pm 0.32$
30	$5.70^{A} \pm 0.21$	$4.30^{B} \pm 0.37$	$2.60^{C} \pm 0.48$	$2.30^{D} \pm 0.33$

Means bearing same superscripts within a row differ significantly (P<0.01)

TABLE 4.61: ANOVA OF SCORES FOR AFTER TASTE IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source of variation	df	SS	MS	F
Treatment	3	323.7188	107.9063	79.88946**
Days	3	10.86875	3.622917	2.682262
Treatment X Days	9	7.10625	0.789583	0.584576^{NS}
Error	144	194.5	1.350694	

4.5.3. vi) Overall acceptability

The mean (±SE) overall acceptability values of chicken chips under different treatment groups having fenugreek leaves are given in Table 4.62 and their analyses of variance in Table 4.63. The overall acceptability scores for chicken chips using spent hen meat incorporated with fenugreek leaves powder are found to be within the range of 2.00 ± 0.33 to 5.50 ±0.34 which indicate 'from very poor to good scores'.

The overall acceptability scores (Mishra *et al*., 2015) of Control and dehydrated chicken meat rings prepared with various extenders did not differ significantly (P >0.05).

The mean (±SE) scores for overall acceptability of chicken chips under different treatment groups having fenugreek seeds are given in Table 4.64 and their analysis of variance in Table 4.65. The analysis of variance revealed significant (P<0.05) differences among the various treatment groups incorporated with fenugreek leaves and Control regarding overall acceptability.

TABLE 4.62: OVERALL ACCEPTABILITY SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control (Without FL)	Treatment I (with 0.25% FL)	Treatment II (With 0.50% FL)	Treatment III (With 1.00% FL)
0	$5.50^{A} \pm 0.34$	$5.00^{B} \pm 0.39$	$3.50^{C} \pm 0.60$	$2.00^{D} \pm 0.33$
10	$5.30^{A} \pm 0.30$	$4.90^{B} \pm 0.35$	$3.60^{C} \pm 0.45$	$2.20^{D} \pm 0.29$
20	$5.50^{A} \pm 0.37$	$4.10^{B} \pm 0.57$	$3.50^{C} \pm 0.45$	$2.40^{D} \pm 0.45$
30	$5.40^{A} \pm 0.31$	$4.60^{B} \pm 0.27$	$3.60^{C} \pm 0.43$	$2.90^{D} \pm 0.43$

Means bearing same superscripts within a row differ significantly (P<0.01)

TABLE 4.63: ANOVA OF SCORES FOR OVERALL ACCEPTIBILITY IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK LEAVES UNDER DIFFERENT TREATMENT GROUPS

Source of variation	*df*	*SS*	*MS*	*F*
Treatment	3	211.85	70.61667	42.65436^{**}
Days	3	1.25	0.416667	0.251678^{NS}
Treatment X Days	9	8.5	0.944444	0.57047^{NS}
Error	144	238.4	1.655556	

TABLE 4.64: OVERALL ACCEPTABILITY SCORES (MEAN ±SE) OF CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Days	**Control (Without FS)**	**Treatment IV (with 0.25% FS)**	**Treatment V (With 0.50% FS)**	**Treatment VI (With 1.00% FS)**
0	$5.60^{A} \pm 0.40$	$4.70^{B} \pm 0.42$	$3.60^{C} \pm 0.45$	$2.10^{D} \pm 0.41$
10	$5.90^{A} \pm 0.38$	$4.40^{B} \pm 0.34$	$2.90^{C} \pm 0.33$	$2.10^{D} \pm 0.35$
20	$5.50^{A} \pm 0.27$	$3.90^{B} \pm 0.38$	$2.50^{C} \pm 0.40$	$2.10^{D} \pm 0.31$
30	$5.70^{A} \pm 0.30$	$4.30^{B} \pm 0.37$	$2.40^{C} \pm 0.34$	$2.30^{D} \pm 0.30$

Means bearing same superscripts within a row differ significantly ($P<0.01$)

TABLE 4.65: ANOVA OF SCORES FOR OVERALL ACCEPTIBILITY IN CHICKEN MEAT CHIPS INCORPORATED WITH FENUGREEK SEEDS UNDER DIFFERENT TREATMENT GROUPS

Source	**df**	**SS**	**MS**	**F**
Days	3	5.45	1.816667	1.37395^{NS}
Treatment	3	296.25	98.75	74.68487**
Treatment X Days	9	7.9	0.877778	0.663866^{NS}
Error	144	190.4	1.322222	

There was significant decrease ($P<0.05$) in certain sensory attributes showing a declining trend in overall acceptability of the treatment groups with increase in level of fenugreek leaves and seeds content. However no changes observed with increase in storage period. The scores for overall acceptability decreased from 5.60 ± 0.40 to 2.10 ± 0.41 for treatment with fenugreek seeds. This might be due to increase in bitterness of the product with the increase in level of fenugreek leaves and/or seeds in the products.

Similar declining trends in sensory attributes could be noticed by Qureshi *et al* (2018) who used fenugreek seeds powder to produce spent hen meat patties. Differing to this observation, Hegazy (2011) concluded that there was no significant difference in color, taste, flavor and appearance scores of beef burger samples with 3 and 6% fenugreek seed flour incorporation.

Singh *et al*. (2011) observed decreasing significant ($P<0.05$) trend of change on the sensory attributes that is, colour and appearance, flavour, texture, crispness, aftertaste, meat flavour intensity and overall acceptability of chicken snacks during the entire storage period of 30 days at ambient temperature 30 ± 2°C.

Such a diminishing but non- significant ($P>0.05$) change was also reported by Sharma *et al*. (2002) in their product spent hen chicken chips in respect of colour, appearance, meat flavour intensity and overall acceptability for a total period of 12 weeks.

Data pertaining to different sensory attributes of chicken meat caruncles such as colour, flavour, crispiness, aftertaste, meat flavour intensity and overall acceptability remained non-significant and were not affected by different levels of baking powder (Singh *et al.,* 2013) during their storage period of 60 days.

All the physicochmical parameters for the treatment groups were found to be within desirable ranges. Among the various treatment groups, the results from the chicken chips prepared with fenugreek leaf (0.25%) and fenugreek seed (0.25%) powder

were found promising. This assessment was based on the observations of proximate analysis, physicochemical and sensory characteristics.

Therefore, in the Phase II of study the chicken chips were prepared with fenugreek leaf powder (0.25%), fenugreek seed powder (0.25%) and a mixture of fenugreek leaf and seed powder (0.25%, each) and compared with Control group as mentioned in the Table 4.66. In the study, the physicochemical and proximate analysis along with sensory, antioxidant, antibacterial properties were evaluated for a storage stability period of 30 days under aerobic condition. The cost of preparation of ready-to-eat chicken chips was also calculated for all the treatment groups to evaluate the economy of production.

4.6. ANALYSIS OF THE TREATMENT GROUPS –PHASE II

TABLE 4.66: FORMULATION OF CHICKEN CHIPS INCORPORATED WITH FENUGREEK LEAVES AND/OR SEEDS POWDER

INGREDIENTS	**PARTS**			
	Control	**Treatment A (with 0.25% FL)**	**Treatment B (With 0.25% FS)**	**Treatment C (With 0.25% FL+FS)**
Meat	60.0	59.75	59.75	59.50
Wheat Flour	10.00	10.00	10.00	10.00
Bengal Gram Flour	12.00	12.00	12.00	12.00
Baking powder	0.50	0.50	0.50	0.50
Corn flour	12.00	12.00	12.00	12.00
Salt	2.00	2.00	2.00	2.00
Spices	1.50	1.50	1.50	1.50
Condiments	2.00	2.00	2.00	2.00
Fenugreek leaves	0	**0.25**	0	**0.25**
Fenugreek seeds	0	0	**0.25**	**0.25**
Total	**100**	**100**	**100**	**100**

The chips were packed in low density polyethylene (LDPE) bag, sealed and stored under aerobic condition up to 30 days. The different physicochemical, microbial,

sensory parameters and storage stability were evaluated at regular period that is on 0^{th}, 10^{th}, 20^{th} and 30^{th} day.

All the samples designated under different treatment groups were analyzed for various proximate, physicochemical properties and sensory evaluation.

4.6.1. Proximate analysis

4.6.1.i) Moisture

The mean (±SE) values of moisture per cent in chicken chips under different treatment groups are given in Table 4.67 and their analysis of variance in Table 4.68. The moisture levels are found to be within the range of 4.36 ± 0.03 to 4.54 ± 0.06 %. Working with spent hen papad, Malav *et al*.(2017) noted the moisture level in between 3.08±0.13 to 12.74±0.05%. Aswathi *et al*. (2013) observed moisture per cent ranged from 6.52 to 7.22% in functional spent hen meat sticks prepared with oat flour and corn flour.

The analysis of variance revealed a significant ($P<0.05$) increase in moisture per cent with increase in storage period. It was interesting to note that there was no significant change in all the treatment groups of chicken chips including Control group from 0 to 20^{th} day of storage irrespective of the levels of herbs (leaf and seed) used. However there was significant ($P<0.05$) increase in the moisture level on 30^{th} days of storage as compared to the 0, 10 and 20^{th} day.

Nonetheless no change in the moisture level was observed among different treatment groups. The increase in moisture content along the storage period might be due to absorption of ambient moisture under aerobic condition. This observation was comparable with results of Devalakshmi *et al.* (2010) who noted significant ($P<0.05$) increase of moisture level in chicken meat chips with progressing storage, both at ambient (37±2°C) as well as at refrigerated (7±1°C) temperatures. The present result is in consonance with the findings of Ahlawat *et al.* (1997) who also observed significantly increased moisture levels in chicken papads stored up to a period of 6 months. Such a finding was also reported by Cakmak *et al*. (2015) while dealing with the product prepared from spent hen meat stored for a period of 90 days at room temperature

(25^0C).This could be accredited to the high moisture binding capacity of muscle proteins of spent hen.

Kumar *et al.* (2016) prepared chicken biscuits using oat bran with a moisture level of 8% in Control and treatment group whereas Talukder and Sharma, (2010) prepared chicken patties with oat bran, where moisture level was found to be 6.0%. Both the groups noted significantly higher moisture level in the treatment groups as compared to Control group. This might be because of the fact that high level of oat, i.e., 3.0, 5.0 and 7% used in the treatment groups. Further, oat bran might have helped for better water retention owing to its high fiber components. In contrary to these works a maximum of 0.5% fenugreek (seed+leaf) powder was used in the present study.

Singh *et al.* (2011) found non-significant increase in the moisture content of the product, meat caruncles for a storage period of 45 days using packaging material (polyethylene LDPE) bags and kept at a storage condition of −18± 1°C. Present findings do not agree with the results of Kalara *et al.* (1987) who worked on vegetable chips using potatoes and found no significant increase of moisture content under ambient storage temperature (30 ^{0}C) for 30 days. The changes noted in the above studies might be due to variation in packaging and storage conditions as mentioned therein.

TABLE 4.67: MEAN (±SE) VALUES OF MOISTURE (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage interval (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (With 0.25% FL+FS)
0	4.36^a± 0.03	4.39^a ± 0.03	4.38^a ± 0.02	4.39^a ± 0.02
10	4.38^a± 0.03	4.38^a ±0.03	4.39^a ± 0.02	4.40^a ± 0.03
20	4.38^a± 0.02	4.40^a ± 0.02	4.40^a ± 0.03	4.38^a ± 0.03
30	4.53^b ±0.04	4.54^b ± 0.02	4.53^b ± 0.01	4.54^b ± 0.06

Means bearing same superscripts within a column differ significantly (P<0.05)

TABLE 4.68 :ANOVA VALUES FOR MOISTURE (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.002554	0.000851	0.186626^{NS}
Days	3	0.327674	0.109225	23.9462*
Treatment x Days	9	0.004641	0.000516	0.11306^{NS}
Error	64	0.29192	0.004561	

4.6.1.ii) Crude protein

The mean (±SE) values of crude protein per cent in chicken chips under different treatment groups are given in Table 4.69 and their analyses of variance in Table 4.70.

From the data presented in the Table 2.51 it can be noted that the crude protein values ranged from 22.36 ± 0.02 to 23.03 ± 0.06%. Berwal *et al*. (1996), while comparing the protein content of turkey meat papads with traditional rice papad found to have comparable values, ranged from 20.35 to 25.75%. Malav *et al*. (2017) evaluated the quality of spent hen meat papad and the crude protein value was found to be quite high (from 17.87±0.08 to 34.09±0.14) as compared to the outcomes of the present study.

The analysis showed a significant (P<0.05) increase in crude protein per cent in Treatment-A, Treatment-B and Treatment-C when compared with Control group. Similar, increase (P<0.05) in protein content was reported by Berwal *et at*. (1996) while preparing turkey meat papad and comparing with traditional rice papads.

Aswathi *et al*. (2013) worked on the effect of different levels of oat flour and corn flour on physicochmical and sensory qualities of poultry meat sticks and found the protein content ranged from 50.82 to 60.51%.

There is significant (P<0.05) increase of crude protein level in Treatment B (22.74±0.04 - 22.93 ±0.05) and Treatment C (22.88 ±0.10 - 23.03± 0.06) as compared to the Treatment A and Control group. This might be due to replacement of meat protein with fenugreek seed power containing higher level of protein (26 per cent).

Further, the days of storage showed no influence on the crude protein content in the chicken chips as evident from the Table 4.69

TABLE 4.69: MEAN (±SE) VALUES OF CRUDE PROTEIN (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage interval (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (With 0.25% FL+FS)
0	$22.37^{A} \pm 0.02$	$22.41^{A} \pm 0.04$	$22.74^{B} \pm 0.04$	$23.03^{C} \pm 0.06$
10	$22.52^{A} \pm 0.03$	$22.39^{A} \pm 0.03$	$22.76^{B} \pm 0.12$	$22.88^{C} \pm 0.10$
20	$22.39^{A} \pm 0.02$	$22.36^{A} \pm 0.02$	$22.93^{B} \pm 0.05$	$23.01^{B} \pm 0.07$
30	$22.46^{A} \pm 0.04$	$22.46^{A} \pm 0.04$	$22.88^{B} \pm 0.03$	$22.99^{C} \pm 0.09$

Means bearing different superscripts within a row differ significantly ($P<0.05$)

TABLE 4.70: ANOVA VALUES FOR CRUDE PROTEIN (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	4.735054	1.578351	29.76126*
Days	3	0.038824	0.012941	0.244019^{NS}
Treatment x Days	9	0.248051	0.027561	0.519693^{NS}
Error	64	3.39416	0.053034	

4.6.1.iii) Ether Extract

The mean (±SE) values of ether extract per cent in chicken chips under different treatment groups are given in Table 4.71 and their analyses of variance in Table 4.72.

The results of statistical analysis of ether extract in chicken chips averaged between 4.65-4.73%. The ether extract values are found to be in the lower range as compared to the results from earlier workers. Bhoyar *et al.* (1996) reported ether extract in the range of 3.8 to 6.5 per cent in chicken steaks. Devalakshmi *et al.*(2010) noted the ether extract values in the range of 7.02 -7.56% in chicken meat chips; whereas Sengar (2014) observed 10.88-22.02% ether extract in pork papad incorporated with natural additives like lemongrass, mint and jimbu at the rate of 0.25, 0.50 and 0.75%. Low ether

extract values in the present study might be due to use low amount of fat rich ingredients in the formulation of chicken chips.

The data analysis showed no significant change (P>0.05) in ether extract per cent due to the four treatments; neither the storage period influenced the parameter.

TABLE 4.71: MEAN (±SE) VALUES OF ETHER EXTRACT (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Days	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (With 0.25% FL+FS)
0	4.65 ± 0.08	4.68 ± 0.02	4.70 ± 0.02	4.71 ± 0.03
10	4.68 ± 0.02	4.68 ± 0.01	4.71 ±0.02	4.73 ± 0.01
20	4.66 ±0.03	4.67 ± 0.05	4.69 ±0.07	4.70 ± 0.02
30	4.65 ± 0.02	4.71 ± 0.02	4.68 ± 0.01	4.73 ±0.02

TABLE 4.72: ANOVA VALUES FOR ETHER EXTRACT (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.37567	0.125223	0.327830^{NS}
Days	3	0.00614	0.002047	0.675468^{NS}
Treatment x Days	9	0.00717	0.000797	0.262926^{NS}
Error	64	0.19392	0.00303	

The result of the crude fat obtained from this study compares favorably with the works of Bhoyar *et al.* (1996) who noted .that per cent ether extract were not significantly affected in chicken steaks prepared using spent hen meat with texturized soy protein at 0, 10, 20 and 30 % levels. Low fat content in foods enhanced shelf life due to less chance of lipid peroxidation.

4.6.1.iv) Total Ash

The total ash content for the product spent hen meat chips prepared in the study are described in the Table 4.73 and the analyses of variance in the Table 4.74. The total ash content of this unique product ranged from 7.41 to 7.47 % after incorporation of fenugreek seeds and leaves powder in the chicken chip formulation. The total ash content found are comparable with the findings of Kasthuri *et al.* (2017) who have reported the value to be within the range of 7.76 ±0.05 to 8.15±0.04. In the present trial there was no significant difference in the total ash content in the chicken chips after incorporation of fenugreek seeds and/or leaves powder among all the treatment groups and Control. Moreover there were also no significant changes in the total ash content with progression of storage period up to 30 days.

Similar study was also noted by Kasthuri *et al.* (2017) on chicken chips added with drumstick leaf and jamun seed powder, wherein no significant alterations were observed up to 30 days storage.

TABLE 4.73: MEAN (±SE) VALUES OF TOTAL ASH (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Days	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment III (With 0.25% FL+FS)
0	7.41± 0.03	7.43 ± 0.01	7.45 ± 0.01	7.44 ± 0.00
10	7.41 ± 0.01	7.42 ± 0.02	7.44 ± 0.02	7.47 ± 0.00
20	7.42 ± 0.01	7.42 ± 0.01	7.44 ± 0.01	7.43 ± 0.01
30	7.42 ± 0.00	7.41 ± 0.02	7.44 ± 0.03	7.45 ± 0.01

Means bearing same superscripts column-wise (Lower case) or row-wise (Uppercase) do not differ significantly ($P<0.05$)

TABLE 4.74: ANOVA OF TOTAL ASH (%) VALUES IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.013845	0.004615	1.792668 ^{NS}
Days	3	0.000765	0.000255	0.099053 ^{NS}
Treatment x Days	9	0.004825	0.000536	0.208249 ^{NS}
Error	64	0.16476	0.002574	

4.6.2. Physico-chemical analysis

4.6.2.i) pH

The pH of meat and meat products is an important measure to estimate relative acidity or alkalinity (Doty, 1960) which might indicate the potential storage life of the product.

The mean ±SE of pH value of ready-to-cook chicken chips using spent hen with addition of fenugreek seeds and fenugreek leaves of the present study are presented in Table 4.75 and analysis of variance in Table 4.76. The pH of all the treatment groups of chicken chips incorporated with fenugreek seeds and/or leaves powder ranged from 5.58 ± 0.05 to 5.69 ± 0.01.

Working with chicken meat rings Soni *et al.* (2013) reported the pH in the range of 5.20-6.18; whereas, Mishra *et al.*(2015) found the pH of their product, dehydrated chicken meat rings to be in the range of 6.13-6.30.

The pH values of all the samples remained comparable with non-significant ($P>0.05$) differences up to 20^{th} day of storage. However on 30^{th} day, significant change in pH values could be recorded in all the treatment groups including that of Control.

Storage days showed significant ($P<0.05$) effect on pH of the dried products. Treatment-C had significantly lower ($P<0.05$) pH on 30^{th} day compared to 0^{th} to 20 day of storage.

TABLE 4.75: MEAN (±SE) VALUES OF pH IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage interval (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (With 0.25% FL+FS)
0	$5.69^{a} \pm 0.03$	$5.63^{a} \pm 0.01$	$5.66^{a} \pm 0.01$	$5.66^{a} \pm 0.01$
10	$5.68^{a} \pm 0.02$	$5.67^{a} \pm 0.01$	$5.67^{a} \pm 0.03$	$5.67^{a} \pm 0.00$
20	$5.69^{a} \pm 0.01$	$5.66^{a} \pm 0.03$	$5.68^{a} \pm 0.02$	$5.67^{a} \pm 0.01$
30	$5.59^{b} \pm 0.06$	$5.55^{b} \pm 0.08$	$5.58^{b} \pm 0.00$	$5.58^{b} \pm 0.05$

Means bearing same superscripts within a column differ significantly ($P<0.05$).

The pH scores showed a decreasing trend during the last part of storage period. This trend was attributed to the chemical activity as hydrolytic rancidity increases free fatty acid level but not to the microbial activity (Mishra *et al.*, 2015). The present findings corroborate well with the findings of Modi *et al.* (2007) who also observed significant decrease in pH while working with dehydrated Kebab mix under ambient storage (27 ± 2 °C) for 6 months.

Such decreasing pH level was also reported by Bennani *et al.*(2000), Rubio *et al.* (2007) and Mishra *et al.* (2015) while working on kaddid, a salted dry mutton; Salchichon, a dried Spanish sausage and dehydrated chicken meat rings, respectively.

As the storage period progressed, the pH values of all samples decreased. Choi *et al.* (2007) reported similar results, wherein the pH of meat products generally decreased during storage. Moreover, the major factor influencing the decrease in the pH was the storage time, and refrigerated storage also caused a decrease in the pH values due to the activity of lactic acid bacteria and dissolution of CO_2 into the pork patties (Rubio *et al.*, 2007).

Kasthuri *et al.* (2017) evaluated the effect of storage on pH of chicken chips incorporated with 1% drumstick leaf powder (DLP), 1% *jamun* seed powder (JSP) and 0.5% DLP + 0.5% JSP stored at room temperature in air tight PET (Polyethylene terephthalate) containers and stored at room temperature for a period of 30 days where significant ($P<0.05$) decline in pH values were observed between the storage days. Another major factor influencing the decrease in the pH level was the storage time, activity of the lactic acid bacteria and dissolution of CO_2 into the meat product (Rubio *et al.,* 2007). Choi *et al.* (2007) in their study found decrease in pH values of stick type reconstructed jerky stored in plastic packaging under room temperature (25^oC) for 90 days.

Contrary to the present study, Devalakshmi *et al*. (2010) investigated that the chicken meat chips added with 15% cooked and mashed potato, 15% bengal gram flour and 15% black gram flour had recorded significantly ($P<0.01$) lower pH values than the Control formulation and the pH tended to increase during the storage period both at ambient (37±2°C) and refrigerated (7±1°C) temperature, irrespective of type of formulation.

Sharma *et al.* (2015) found the pH value of the product, chicken snack utilizing 10% extenders like potato (boiled and mashed) and black gram (*Vigna mungo*) flour in 11 ratio to be in significant ($P<0.05$) increasing trend with increase in storage.

TABLE 4.76: ANOVA OF VALUES FOR pH IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.007104	0.002368	0.987146 ^{NS}
Days	3	0.009644	0.003215	1.340108 *
Treatment x Days	9	0.006581	0.000731	0.304846 ^{NS}
Error	64	0.15352	0.002399	

4.6.2.ii) Tyrosine value

The mean ±SE of tyrosine value of ready-to-cook chicken chips using spent hen with addition of fenugreek seeds and/or fenugreek leaves are presented in Table 4.77 and analysis of variance in Table 4.78.

TABLE 4.77: MEAN (±SE) VALUES OF TYROSINE (mg/100g) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage interval (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FL)
0	5.10 ± 0.07	4.95 ± 0.06	5.09 ± 0.15	5.07 ± 0.18
10	5.17 ± 0.02	5.09 ± 0.04	5.15 ± 0.05	5.08 ±0.04
20	5.04 ± 0.04	5.04 ± 0.03	5.16 ± 0.06	5.10 ± 0.06
30	4.39 ± 0.07	5.06 ± 0.03	5.15 ± 0.02	5.10 ±0.03

TABLE 4.78: ANOVA OF TYROSINE VALUES (mg/100g) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.085777	0.028592	0.815668 ^{NS}
Days	3	0.098001	0.032667	0.931908 ^{NS}
Treatment x days	9	1.577421	0.175269	9.95776^{NS}
Error	64	1.12648	0.017601	

It has been observed that the tyrosine values ranged from 4.95 ± 0.06 to 5.17 ± 0.02%. The values obtained are comparable with the earlier reports of Kasthuri *et al.* (2017) who noted the values of 4.43±0.29 to 7.63±0.42% in chicken chips using drumstick leaf and jamun seed powder. However, the analysis of variance revealed no significant difference (P>0.05) in tyrosine value among the treatment groups and during the storage periods.

Tyrosine value is an indicator of proteolysis as it measures the levels of tyrosine and tryptophan in the extract of meat. In the current study, the ready-to-cook chicken chips are dehydrated meat products and there was minimal chance of microbial growth. The absence of microbial proteolytic activity might have prevented to occur any kind of change in the tyrosine value of the product stored up to 30 days.

The findings of the present study was supported by the reports of Kasthuri *et al.* (2017) which showed no significant differences in tyrosine value up to 20^{th} day of storage. There was no significant difference among the treatment groups throughout the storage study and the values remained far below permissible limit for all the products i.e. 35-80mg/100g (Naik *et al.,* 2015).

Contrary to the current findings, Karthikeyan *et al.* (2010) reported significant increase in tyrosine value (P<0.05), during storage of buffalo meat extruded product upto 60 days.

4.6.2.iii) Water activity (a_w)

The mean ±SE of water activity values of ready-to-cook chicken chips using spent hen meat with addition of fenugreek seeds and/or fenugreek leaves are recorded at 10 days interval for 30 days of storage and are presented in Table 4.79 and analysis of variance in Table 4.80. The water activity of the chicken chips ranged from 0.683 to 0.716 which are comparable with the findings of Salgeuro *et al.* (1994) who recorded water activity from 0·60 to 0·91 in intermediate-moisture meat products stored under ambient condition (25^0C) for three months.

The analysis of variance revealed significant difference (P<0.05) in water activity values on 30th day of storage compared to the a_w on the day of preparation and 10th and 20th day of storage. The water activity ranged from 0.684-0.685 from 0th to 20th day of storage whereas significant increase in water activity was found on 30th day, which ranged from 0.705 ± 0.01 to 0.716 ± 0.00. No significant changes in water activity parameter were found in all the treatment groups including the Control group, stored up to 20th day.

TABLE 4.79: MEAN (±SE) VALUES OF WATER ACTIVITY IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage Days	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FL)
0	0.684^{a} ± 0.00	0.683^{a} ± 0.00	0.689^{a} ± 0.00	0.690^{a} ± 0.00
10	0.684^{a}± 0.00	0.684^{a}± 0.01	0.688^{a} ± 0.01	0.688^{a} ± 0.01
20	0.685^{a}± 0.01	0.682^{a}±0.01	0.690^{a} ± 0.00	0.688^{a} ± 0.01
30	0.713^{b} ± 0.00	0.705^{b} ± 0.01	0.716^{b} ± 0.00	0.715^{b} ± 0.01

Means bearing different superscripts within a column differ significantly (P<0.05)

TABLE 4.80: ANOVA OF VALUES FOR WATER ACTIVITY IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.000677	0.000226	1.730645 ^{NS}
Days	3	0.009705	0.003235	24.79347*
Treatment X Days	9	0.000129	1.44E-05	0.110063 ^{NS}
Error	64	0.008351	0.00013	

In common, drying is the lowering of a_w of perishable products accomplished by removing water, which restricts the growth of micro-organisms (Thiagarajan, 2008). Nevertheless, the temperature and relative humidity of the environment and the characteristics of the packaging material used are also important (Simal *et al.,* 2003) to determine the water activity of the product.

In the present trial, the water activity (a_w) showed no significant difference (P>0.05) between Control and four treatment groups up to 20th day of storage. This might be related with the fact that there was increase in moisture per cent on the 30th day of storage for the chicken chips (Table 4.67). With the increase in moisture content of the product, it might be responsible for the changes in the water activity values of the product.

The water activity is an inherent property of foods indicating the amount of water available to microbes for their growth and is directly responsible for microbiological safety of the foods (Gibbs and Gekas, 2010).

The high or low water activity value can also be influenced by cooking temperature (Surbakti *et al.,* 2016), drying temperature and time, and storage time (Asgar *et al*., 2010). The lower water activity of the chicken chips on the day of preparation was reflected in the lower moisture content of the product and higher water activity of chicken chips product reflects higher moisture content due to storage. The results are in agreement with that of Thomas *et al*. (2006), who observed a lower water activity with lowering of moisture content in sausage under ambient temperature (37±1°C) storage. Lewicki (2004) observed lower water content in Nigeraean soup and was related to lowered water activity, stored using canning and freezing techniques.

4.6.2.iv) Cooking yield

TABLE 4.81: MEAN (±SE) VALUES OF COOKING YIELD (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Days	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FL)
0	94.05 ± 0.60	93.91 ± 0.19	94.19 ± 0.53	94.15 ± 0.26
10	93.92 ± 0.88	94.18 ± 0.18	94.00 ±1.01	94.00 ± 0.59
20	94.12 ± 0.38	94.09 ± 0.14	94.00 ±0.53	94.17 ± 0.33
30	93.91 ± 0.26	93.94 ± 0.18	94.12 ± 0.28	93.86 ± 0.33

TABLE 4.82: ANOVA OF VALUES FOR COOKING YEILD (%) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	Df	SS	MS	F
Treatment	3	0.07528	0.025093	0.015783^{NS}
Days	3	0.16294	0.054313	0.034161^{NS}
Treatment X Days	9	0.53462	0.059402	0.037362^{NS}
Error	64	101.7542	1.589909	

In the current study, the mean ±SE of cooking yield per cent of ready-to-cook chicken chips using spent hen meat with addition of fenugreek seeds and/or fenugreek leaves are presented in Table 4.81 and analysis of variance in Table 4.82. In order to determine the cooking yield, the chicken chips were exposed to baking temperature 100^0C under Microwave Oven using cooking liquid (Vegetable oil) with the help of brush.

The values obtained for cooking yield ranged from 93.86 ± 0.33 to 94.17 ± 0.33% in all the treatment groups including the Control group. It was interesting to find that the cooking yields obtained from the present study are in the higher range than the report of earlier workers. Cytyarasan (2011) noted the yield ranging from 58.95 to 73.87%. However, there have been no significant (P>0.05) differences in the cooking yield per cent of the chicken chips prepared from spent hen among the treatment groups and Control. The high yielding values of the chicken chips might be due to the use of microwave technique of cooking with low use of cooking oil. There was no significant (P>0.05) change in cooking yield of the treatment groups with increase in storage period.

Kasthuri *et al*. (2017) studied the effect of incorporating 1% drumstick leaf powder (DLP), 1% *jamun* seed powder (JSP) and 0.5% DLP + 0.5% JSP at room temperature for a period of 30 days and found no significant differences among the treatment groups. Similarly, Najeeb *et al*.(2015) reported that incorporation of DLP at 1% level had no significant effect on cooking yield of restructured chicken blocks. Chand *et al*. (2013) recorded the cooking yield of 24.59 - 26.72% in ready-to-fry chicken meat chips. Cytyarasan (2011) reported no significant difference in cooking yield (58.95 – 73.87%) of murukku with different levels of chicken skin powder.

Fenugreek leaves and seed powder could improve the hydration and cooking yield of the meat product but in the present study it could not significantly express, which might be due to incorporation of lower level of fenugreek powder in the meat product.

Similar to the present findings, significant improvement ($P<0.05$) in cooking yield per cent could be observed in the results of experiments conducted by Hegazy (2011) who developed a product, beef burger containing fenugreek seed flour at the levels of 9 and 12%. Contrary to this, Acton and Dick (1978) recorded lower cooking yield in the turkey meat loaves while using skin at the level of 10-50% and cooking at 78^0 C. Buyck *et al.* (1982) prepared chicken patties from spent hen containing 0, 10, 20 and 30% adding skin and fat and found low cooking yield. The poor water binding capacity of this product and high fat incorporation are attributed for low cooking yield.

4.6.3. Storage stability under aerobic packaging up-to 30 days

4.6.3.i) Thiobarbituric acid number (TBA)

Any meat product with lipid content has to be estimated for oxidative rancidity in order to determine its storage stability. Oxidation of lipid tends to cause spoilage of the product with the development of off flavour. Thiobarbituric acid values are useful to determine the carboxyl residues in the form of malondialdehyde (MDA) formation which breaks down the products by lipid peroxidation. The degree of spoilage and rancid change depend on the other factors such as physico-chmical change, oxygen availability, storage temperature and bacterial contamination.

The mean of Thiobarbituric acid values of ready-to-cook chicken chips using spent hen with addition of fenugreek seeds and/or fenugreek leaves are presented in Table 4.83 and analysis of variance in the Table 4.84. On the day of preparation of the product the value remained within the range from 0.28 ± 0.01 to 0.34 ± 0.02 mg malondialdehyde/kg. The TBA value significantly ($P<0.05$) decreased on the 10^{th}, 20^{th}, and 30^{th} day of storage. However, the values remained comparable beyond 10^{th} day of study. The increase of TBA values was generally an indicator of lipid peroxidation (Xiong *et al.*, 2020).

TABLE 4.83: MEAN (±SE) VALUES OF TBA (mg malondialdehyde/kg) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Days	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	$0.34^{a} \pm 0.02$	$0.32^{a} \pm 0.02$	$0.28^{a} \pm 0.01$	$0.30^{a} \pm 0.01$
10	$0.23^{b} \pm 0.01$	$0.25^{b} \pm 0.02$	$0.24^{b} \pm 0.01$	$0.25^{b} \pm 0.00$
20	$0.27^{b} \pm 0.01$	$0.26^{b} \pm 0.01$	$0.26^{b} \pm 0.01$	$0.27^{b} \pm 0.01$
30	$0.29^{b} \pm 0.01$	$0.28^{b} \pm 0.01$	$0.28^{a} \pm 0.00$	$0.28^{b} \pm 0.00$

Means bearing different superscripts within a column differ significantly ($P<0.05$)

TABLE 4.84: ANOVA OF VALUES FOR TBA (mg malondialdehyde/kg) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.002405	0.000802	0.775025^{NS}
Days	3	0.049665	0.016555	16.00483*
Treatment X Days	9	0.008125	0.000903	0.872776^{NS}
Error	64	0.0662	0.001034	

Kasthuri *et al*. (2017), in their product chicken chip incorporated with drumstick and jamun seed powder found to have TBA values ranging from 0.14±0.01 to 0.34±0.02 mg MDA/kg up to 30 days of storage under tight PET containers. Biswas *et al.* (2014) recorded the TBA value of 0.24-1.24 mg MDA/kg in poultry meat wafers stored up to 60 days under ambient temperature (25 ± 2^{0}C).

The TBA value on first day might be due to the processing techniques involved in the preparation of the chicken chips such as mincing and mixing that might have resulted in widespread cellular destruction of meat (Rhee and Myers, 2003). This contributed to occurrence of lipid oxidation (Kasthuri *et al.*, 2017). Exposing of meat product to high temperature during drying process might also lead to an increase of TBA values. The TBA values are duly dependent on the storage time (Hoac *et al*.,2006). But in the present experiment, no significant changes in the TBA values were obtained

among the treatments and Control products. The values however found to be much below the threshold level of oxidative spoilage of the products. Therefore, it may be claimed that the fenugreek seeds, leaves and other spices have antioxidant property when added to the prepared product. Hwang *et al.* (2013) and Nieto *et al.* (2017) showed similar consequences in TBA values in chicken nuggets and sausages respectively with addition of herbs to increase the shelf life. Although, TBA values increased gradually with increasing storage period, the values were within the acceptable limit of 1-2 mg malonaldehyde/ kg meat (Watts, 1962)

Dissimilar results were obtained by Devalakshmi *et al.* (2010) where the overall mean TBA values of chicken meat chip formulations added with 15% Bengal gram flour were significantly ($P<0.01$) higher than the Control. Another such finding of Janardhana Rao (1997) in chicken meat loaves was noted with significant increase in TBA values during the ambient (37±2°C) and refrigerated (7±1°C) storage. Sharma *et al.* (2015) in their study developed an innovative ready-to-eat chicken snack and the TBA value differed significantly ($P<0.05$) for Control and treatment products during storage period. Biswas *et al.* (2014) recorded the TBA value of (0.24-1.24 mg malonaldehyde/kg) in poultry meat wafers and found that TBA value showed increasing trend throughout the storage period of 60 days at ambient temperature.

There was no significant difference in TBA value among the chicken chips prepared by Kasthuri *et al*. (2017) on all the days of storage. TBA value was found to be higher on 1st day and it showed decreasing trend till 20th day of storage followed by significant ($P<0.05$) increase on 30th day of storage at room temperature.

The TBARS value of dehydrated chicken meat rings increased significantly ($P<0.05$) on 15^{th} day of storage as compared to initial value and thereafter it remained comparable up to 30^{th} day of storage and then a non-significant ($P>0.05$) decrease in TBARS value was observed on 45th day of storage (Mishra *et al.* 2015). Cytyarasan (2011) reported that TBA value of murukku incorporated with 10% raw chicken skin and 7.5% chicken skin powder increased significantly ($P<0.01$) during the storage at room temperature.

4.6.4. Assay for cholesterol per cent

The mean of per cent cholesterol of ready-to-cook chicken chips using spent hen with addition of fenugreek seeds and fenugreek leaves are presented in Table 4.85 and analysis of variance in the Table 4.86. The results obtained are as 3.55 ± 0 .14, 3.45 ±0.21, 3.39 ± 0.16 and 3.44 ± 0.14 for Control, Treatment-A, Treatment-B and Treatment -C, respectively.

TABLE 4.85: MEAN (±SE) VALUES OF CHOLESTEROL (mg/100g) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Parameter	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
Cholesterol	30.55 ± 0 .14	30.45 ± 0.21	30.39 ± 0.16	31.44 ± 0.14

TABLE 4.86: ANOVA OF VALUES FOR CHOLESTEROL (mg/100g) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	0.063055	0.021018	0.146131^{NS}
Error	16	2.30132	0.143833	

Lee *et al.* (2003) in their product popped snacks prepared with spent hen meat recorded cholesterol values of 38-52 mg/100g , Jin *et al*. (2007) prepared sausage containing spent laying hen surimi recorded cholesterol content of about 32- 40 mg/100g.

The ready-to-cook chicken meat chips made from spent hen recorded no significant (P>0.05) cholesterol values when compared to the Control without addition of fenugreek. These findings are also in agreement with Ang and Hamm (1982), Jin *et al.* (2007) and Trindade *et al.* (2004) who reported lower level of cholesterol in products prepared using spent hen meat. In the present study, presence of fenugreek leaves and

seeds in the chicken chips did not show any effect on the cholesterol content of the product. This might be due to addition of very less concentration of herbs in the product. In the findings of Gupta and Sharma (2017), cholesterol content of chevon patties with addition of oats and spent hen meat slices respectively lowered the cholesterol level.

4.6.5 Colour profile analysis

TABLE 4.87: MEAN (±SE) VALUES OF COLOUR PROFILE (L*, a*, b*) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Parameter	Control	Treatment A (With 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
Lightness (L*)	61.99^{A}±1.34	54.68^{B}± 1.45	55.77^{BC}± 0.99	56.0^{C} ± 1.58
Redness/Greenness (a*)	7.45 ±0.21	7.69 ± .169	8.65 ± 0.73	7.84 ± 0.21
Yellowness/Blueness (b*)	27.45 ± 1.03	25.68 ± 1.17	27.54 ± 1.71	26.28 ± 1.17

Means bearing different superscripts within a row differ significantly (P<0.05)

TABLE 4.88: ANOVA FOR COLOUR PROFILE (L*, a*, b*) IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Parameter	Source of Variation	df	SS	MS	F
Lightness (L*)	Treatment	3	1.64	0.54	4.50*
	Error	16	1.94	0.12	
Redness/Greenness (a*)	Treatment	3	6724.80	2241.60	0.11^{NS}
	Error	16	335618.00	20976.12	
Yellowness/Blueness (b*)	Treatment	3	3.02	1.00	0.17^{NS}
	Error	16	92.67	5.79	

The mean (±SE) values of lightness (L*) of the prepared chicken chips under different treatment groups are given in Table 4.87 and their analyses of variance in Table 4.88.

The L* values of the products ranged from 54.68 ± 1.45 to 61.99 ± 1.34 and showed decrease in values with the addition of fenugreek seed and/or leaves powder. The results revealed significantly higher values for lightness ($P<0.05$) in the Control group than the other treatment groups. The values of lightness in treatments observed to be on lower side than the Control might probably be due to the presence of fenugreek seeds and leaves powder in them when compared to Control. The reduction in lightness values could have been due to the colouration of fenugreek powder that was used in preparation of chicken chips. The results of the present study was supported by Zaki (2018) where the control samples had higher L* values than the other sausage samples incorporated with fenugreek seed powder but no significant differences were found between the treated sausage samples. Hawashin *et al*. (2016) who prepared beef burgers with addition of destoned olive cake powder caused darkening of beef burgers. Essid *et al.* (2018) prepared ewe sausages adding rosemary powder and noted reduce in lightness (L*) value in the product.

There was no significant difference ($P>0.05$) in the Redness/Greenness (a*) value between Control and treatment groups with addition of fenugreek seeds and/or leaves powder as well as among the treatments. The redness (a*) value of the chicken chips with the addition of fenugreek seeds showed the value ranging from 8.65 ± 0.73 to 7.45 ±0.21 with numerically highest a* value in the treatment group with fenugreeks seeds. In the current experiment with chicken meat chips, the values for yellowness ranged from 25.68 ± 1.17 to 27.54 ± 1.71 and showed no significant difference ($P>0.05$) between treatments and Control. Yellowish colours of the product also indicate the freshness of chicken or processed products (Patriani and Apsari, 2021).

Chicken based dehydrated meat products have comparatively lower values for redness (a*), yellowness (b*) and chroma than chevon, mutton and pork based dehydrated meat products (Meshram *et al.*, 2012).

The methods of drying have significant effect on the lightness (L*), redness (a*) and yellowness (b*) values of any meat product which reflect the degree of browning during drying as well as being a cause of variation in light scattering from the surface of the meat (Van Oeckel *et al*., 1999).

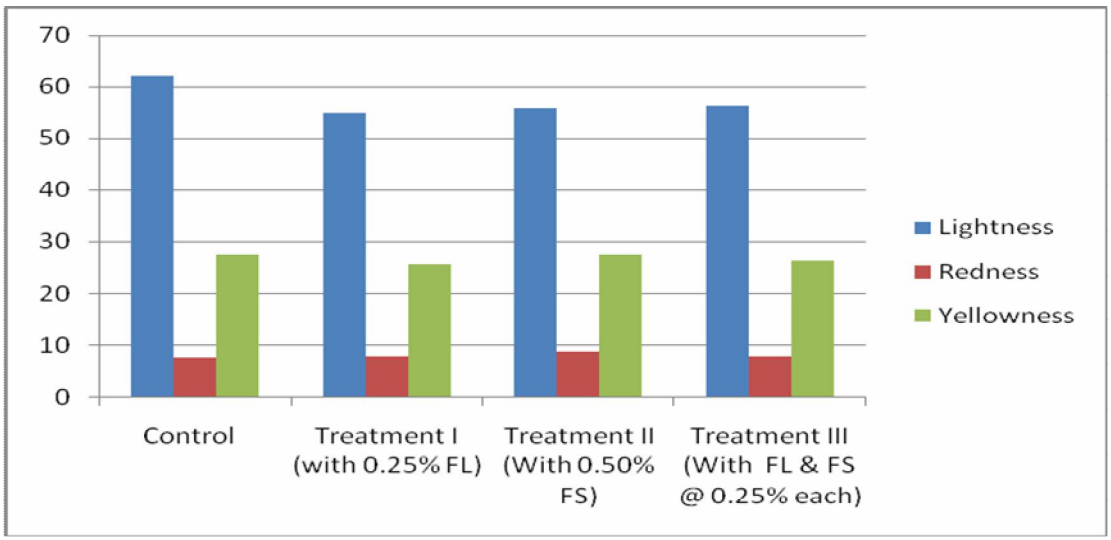


FIG. 4.9: GRAPHICAL REPRESENTATION OF COLOUR PROFILE OF CHICKEN CHIPS

4.6.6. Antioxidant testing assays

4.6.6. i) DPPH radical scavenging activity

The mean (±SE) values of DPPH per cent radical scavenging activity of the prepared chicken chips under different treatment groups are given in Table 4.89 and their analyses of variance in Table 4.90.

In the present work, the mean DPPH per cent radical scavenging activity were 17.03 ± 0.24, 20.41 ± 0.73, 20.89 ± 0.24 and 20.86 ± 0.31 for Control, T-A, T-B and T-C groups, respectively. There was an increase ($P<0.05$) in DPPH radical scavenging activity values with increase addition of fenugreek seeds or leaves powder in all the treatment groups as compared to Control group. Moreover a significant increase in DPPH activity noted with increase in concentration. The increase in the values with increasing levels of fenugreek powder might be attributed to the better antioxidant potential of phenolic rich fenugreek seed and leaves. Adoms *et al.* (2005) showed that the polyphenols were the essential for inhibiting the oxidative damage and were important contributors in determining the antioxidant capacity. The inhibition percentage represents the antioxidant activity on the DPPH solution. The per cent of inhibition was resulted from the number of free radicals that was neutralized by antioxidant contained in the samples.

TABLE 4.89: THE MEAN (±SE) PER CENT DPPH RADICAL SCAVENGING ACTIVITY IN CHICKEN CHIPS INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

Concentration	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
50	17.03^{aA}±0.26	20.41^{bA} ± 0.25	20.89^{bA} ± 0.64	20.86^{bA}±0.46
100	18.00^{aB}±0.27	20.75^{bA} ± 033	21.27^{bB}± 0.43	21.96^{cB}±0.31
150	18.75^{aB}±0.35	21.03^{bB} ± 0.52	21.37^{bB}±0.28	22.65^{cC}±0.38
200	19.62^{aC} ±0.62	21.96^{bC} ± 0.18	22.07^{bC} ± 0.35	22.72^{cC}±0.29
MEAN	17.03^{A} ± 0.24	20.41^{B} ± 0.73	20.89^{C} ± 0.24	20.86^{C}±0.31

Means bearing different superscripts within a row (Lower-case) and within a column (Upper-case) differ significantly (P<0.05)

Similar findings were obtained by Qureshi *et al*. (2018) in spent hen meat patties where significant increase in DPPH radical scavenging activity were recorded with increase in levels of fenugreek seed powder. Ishtiaque *et al*. (2015) conducted a study on antioxidant activity of fenugreek and found its potential antioxidant capacity.

TABLE 4.90: ANOVA FOR PER CENT DPPH RADICAL SCAVENGING ACTIVITY IN CHICKEN CHIPS INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

Source of Variation	df	SS	MS	F
Treatment	3	6.85159	2.283863	19.27019*
Concentration	3	31.79095	10.59698	89.41246*
Error	9	1.066662	0.118518	

4.6.6 ii) Total Phenolic Content

The mean (±SE) values for Total Phenolics content of the prepared chicken chips under different treatment groups are given in Table 4.91 and their analyses of variance in Table 4.92.

The antioxidant activity of chicken chips prepared using spent hen meat incorporated with fenugreek seeds and/or leaves at a concentration of 50µg/ml was found to be 1.840 ± 0.13 in Control, 1.912 ± 0.14 in Treatment- A, 1.929 ± 0.16 in Treatment-

B and 1.954 ± 0.23 in Treatment- C. At the concentration of 100µg/ml, the total phenolic content was found to be 1.887± 0.09 in Control, 1.924 ± 0.16 in Treatment- A, 1.897 ± 0.11 in Treatment- B and 1.979 ± 0.19 in Treatment- C. At the concentration of 150µg/ml, the total phenolic content was found to be 1.890± 0.12 in Control, 1.902 ± 0.11 in Treatment- A, 1.930 ± 0.17 in Treatment- B and 1.947 ± 0.16 in Treatment- C. At the concentration of 200µg/ml, the total phenolic content was found to be 1.897 ± 0.14 in Control, 1.924 ± 0.08 in Treatment- A, 1.944 ± 0.21 in Treatment- B and 1.971 ± 0.10 in Treatment- C. The mean antioxidant activity of chicken chips in terms of total phenolic content are recorded as 1.890 ± 0.27 in Control, 1.916 ± 0.13 in Treatment- A, 1.925 ± 0.34 in Treatment- B and 1.963 ± 0.13 in Treatment- C.

The total phenolic content of the chicken chips were significantly higher in the treatment groups when compared with the Control. The Treatment-C has the highest phenol content value. The higher the phenol content in the product showed an increase in antioxidant activity. The phenolics present in the natural antioxidants have strong H·-donating activity (Muchuweti *et al.*, 2007) or have high radical-absorbance capacity. Some phenolics prevent the formation of free radicals, whereas other scavenge free radicals and chelate prooxidants (Ozsoy *et al.*, 2009). The antioxidant activity may be the result of the synergistic action of all the components, rather than a single entity of the extract.

TABLE 4.91: THE MEAN ± SE TOTAL PHENOLICS CONTENT (GAE/g) PRESENT IN CHICKEN CHIPS INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

CONCENTRATION (µg/ml)	Control	Treatment A (With 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
50	1.840^{aA}±0.13	1.912^{aB} ± 0.14	1.929^{aB} ± 0.16	1.954^{aC}± 0.23
100	1.887^{bA}±0.09	1.924^{aB} ± 0.16	1.897^{bA}± 0.11	1.979^{bC}± 0.19
150	1.890^{bA}±0.12	1.902^{aB}± 0.11	1.930^{aC} ± 0.17	1.947^{aD}± 0.16
200	1.897^{bA}±0.14	1.924^{aB} ± 0.08	1.944^{bC} ± 0.21	1.971^{bD}± 0.10
MEAN	1.890^{A}± 0.27	1.916^{B} ± 0.13	1.925^{B} ± 0.34	1.963^{C} ± 0.13

Means bearing different superscripts within a row (Upper-case) and within a column (Lower case) differ significantly (P<0.05)

TABLE 4.92: ANOVA FOR TOTAL PHENOLICS CONTENT (GAE/g) IN CHICKEN CHIPS INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

Source of Variation	df	SS	MS	F
Treatment	3	0.001352	0.000451	1.422237*
Concentration	3	0.014303	0.004768	15.04742*
Error	9	0.002852	0.000317	

4.6.7. Microbiological studies

4.6.7.i) Total plate count

The chicken chips incorporated with fenugreeks seed or leaves powder were subjected for Total plate count (TPC log 10 cfu/g) on day zero and subsequently at 10^{th}, 20^{th} and 30^{th} day of storage under aerobic condition packed under LDPE bag. The mean values of Total plate count (TPC) of chicken chips are presented in Table 4.93 and the analysis of variance given in Table 4.94.

TABLE 4.93: MEAN ± SE TOTAL PLATE COUNT (cfu/g) OF CHICKEN CHIPS AT DIFFERENT LEVELS OF FENUGREEK SEEDS AND/OR LEAVES UNDER AEROBIC CONDITION

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	ND	ND	ND	ND
10	ND	ND	ND	ND
20	ND	ND	ND	ND
30	1.74^{a} ± 0.08	1.52^{ab}± 0.04	1.48^{b} ± 0.07	1.42^{bc} ± 0.04

ND= Not detected)
Means bearing different superscripts within a row differ significantly (P<0.05)

TABLE 4.94: ANOVA FOR TOTAL PLATE COUNT (cfu/g) OF CHICKEN CHIPS AT DIFFERENT LEVELS OF FENUGREEK SEEDS AND/OR LEAVES UNDER AEROBIC CONDITION

Storage interval (days)	Source of Variation	df	SS	MS	F
0	Treatment	-	-	-	-
	Error	-	-	-	-
10	Treatment	-	-	-	-
	Error	-	-	-	-
20	Treatment	-	-	-	-
	Error	-	-	-	-
30	Treatment	0.4495	3	0.149833	0.227509*
	Error	7.903	12	0.658583	

There was no evidence of viable microbial growth in the chicken chips up to 20^{th} day of storage under aerobic packaging. This presented the sanitary and hygienic conditions maintained during all the processing steps and no significant post-processing contamination was observed. The data analysis described that there was an apparent growth of microorganisms on 30^{th} day in all the treatment groups along with the Control group. The TPC values obtained was significantly high in Control when compared with Treatment B and Treatment C. Whereas, no significant difference was confirmed between the treatment groups. Higher TPC values might be attributed due to the effect of moisture, pH, water activity and availability of oxygen forming a favourable condition for the growth of the microorganisms. Addition of fenugreek might have restricted in the growth of microorganisms in the treatment groups due to the antimicrobial property of the fenugreek.

However, the total plate count values remained below the permissible limit, i.e. $\log 10^7$ cfu/g for cooked meat products (Jay, 1996).

The growth of microorganism was slow and within limit. This might be due to lower water activity of the products (Table 4.79). The increase in TPC during storage of meat products was also reported by Panov and Lubanetskii (1979).

Ajina *et al.* (2012) attributed the lower bacterial growth exhibited in fenugreek leaves groups over storage to antimicrobial compounds present in crude fenugreek leaves which could degrade the wall of cell, damage the cytoplasmic membrane, disrupt membrane proteins and interfere with membrane integrated enzymes, and consequently lead to death of cell. Additionally, crude fenugreek leaves could influence the pH of meat product as well as its content of steroids compound and volatile oil such as tannins and flavonoids that reduce or inhibit microbial activity and biochemical reactions that cause deteriorative changes and spoilage through enzymatic, chemical and physical activities.

Similar observations were found by Sharma *et al.* (2015), who evaluated microbiological growth in ready-to-eat chicken snack for 15 days and was found that the product was quite desirable and acceptable up to 15 days of storage without any marked deterioration in the quality. Similar results were obtained by Verma (2008) in designer chicken nuggets. The results of the present study are in agreement with that of Singh *et al.* (2009) and Mishra *et al.* (2015) who also reported an increase in total plate counts in aerobically packed chicken snacks stored at ambient temperature. Soni *et al.* (2013) and Mishra *et al*. (2015) reported increasing trend in TPC (log cfu/g) of the dehydrated meat rings with advancement of storage days at ambient temperature. Cytyarasan *et al*. (2011) observed TPC count of murukku, incorporated with 10% raw chicken skin and 7.5% chicken skin powder ranged from 2 to 3.13 log cfu/g and the counts increased significantly ($P<0.05$) during the storage up to 30 days at room temperature.

4.6.7.ii) Test for Coliform bacteria

TABLE 4.95: INCIDENCE OF COLLIFORM BACTERIA IN CHICKEN CHIPS AT DIFFRENET LEVELS OF FENUGREEK SEED AND LEAVES UNDER AEROBIC CONDITION (MEAN ± SE)

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	ND	ND	ND	ND
10	ND	ND	ND	ND
20	ND	ND	ND	ND
30	ND	ND	ND	ND

(ND= Not detected)

The coliform bacteria tend to grow well at temperature 37^0 C (Jay, 2000) and can easily grow in meat products stored at ambient temperature. The pathogenic bacteria under the coliform group such as *E coli* are mostly responsible for causing infections and food poisoning harmful for humans (Jaja *et al.,* 2018).

In the present work, the chicken chips incorporated with fenugreeks seed or leaves powder were subjected for coliform bacterial count on day zero and subsequently at 10^{th}, 20^{th} and 30^{th} day of storage under aerobic packing. The mean values of coliform bacterial count of aerobically packed chicken chips using spent hen meat are presented in Table 4.95. The coliform bacteria was not detected in chicken chips incorporated with fenugreek seeds and/or leaves powder throughout the storage study of 30 days under aerobic condition.

Similarly Cakmak *et al.* (2015) in their study observed no significant ($P>0.05$) changes in coliform bacteria count in the snacks using chicken meat and chicken meat powder, both at the beginning (day 0) and at the end of the storage (90^{th} day). Das and Jayaraman (2003) also reported absence of coliforms during ambient temperature storage of dehydrated chicken pulav up to 8 months.

Absence of coliform indicated that proper maintenance of sanitary and hygienic procedures were taken into account during the processing of the meat and preparation of chicken chips. Hence the ready-to-cook chicken chips can be effectively stored up to 30 days and considered safe for human consumption.

4.6.7.iii) Test for Staphylococcal bacteria

TABLE 4.96: INCIDENCE OF STAPHYLOCOCCAL BACTERIA IN CHICKEN CHIPS AT DIFFRENET LEVELS OF FENUGREEK SEED AND LEAVES UNDER AEROBIC CONDITION (MEAN ± SE)

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	ND	ND	ND	ND
10	ND	ND	ND	ND
20	ND	ND	ND	ND
30	ND	ND	ND	ND

(ND= Not detected)

The Staphylococcal bacteria are recognized as one of the major food-borne pathogens in meat and meat products (Wu *et al.,* 2018). The bacteria produce a wide range of extracellular toxins which are mostly heat stable proteins responsible of causing illness to human beings (Hassan *et al.*, 2018). In the present study, the chicken chips incorporated with fenugreeks seed or leaves powder were subjected for Staphylococcal bacterial count on 0^{th}, 10^{th}, 20^{th} and 30^{th} day of storage under aerobic packing. The Staphylococcal bacterial count of aerobically packed chicken chips made of spent hen meat are presented in Table 4.96. In this study, no evidence of Staphylococcal counts was detected during storage period of 30 days in all the treatment groups including Control group. It may be due to lower water activity in the product and hygienic handling and packaging steps followed. This indicated that the product was free from any kind of Staphylococcal contamination and was safe for human consumption.

4.6.7.iv) Test for Salmonella

The presence of Salmonella in poultry meat and meat products occurs primarily due to cross-contamination and under-cooking (Luber, 2009). Detection of this pathogen in poultry meat product both at the production level and before consumption can play a significant role in the prevention of food borne salmonellosis (Temelli *et al.*, 2012). In the present study, tests were conducted for the detection of Salmonella in the chicken chips at 10 days interval up to 30 days storage period and the results are presented in the Table 4.96.

TABLE 4.97: INCIDENCE OF SALMONELLA IN CHICKEN CHIPS AT DIFFRENET LEVELS OF FENUGREEK SEED AND LEAVES UNDER AEROBIC CONDITION (MEAN ± SE)

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	ND	ND	ND	ND
10	ND	ND	ND	ND
20	ND	ND	ND	ND
30	ND	ND	ND	ND

(ND= Not detected)

The incidence of Salmonella was not detected in chicken chips incorporated with fenugreek seeds and/or leaves powder throughout the storage period of 30 days under aerobic condition. This indicated that proper hygienic procedures were followed during the processing and preparation of the products and thus can be considered safe for consumption.

4.6.8.v) Test for presence of Yeast and Mould Count

TABLE 4.98: MEAN ± SE YEAST AND MOULD COUNT (cfu/g) IN CHICKEN CHIPS AT DIFFRENET LEVELS OF FENUGREEK SEED AND LEAVES UNDER AEROBIC CONDITION

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	ND	ND	ND	ND
10	ND	ND	ND	ND
20	ND	ND	ND	ND
30	ND	ND	ND	ND

(ND= Not detected)

The yeast and mould count of aerobically packed chicken chips made of spent hen meat are presented in Table 4.98. From the present study, it was fascinating to know that, there was no detection of yeast and mould in the chicken chips product stored at ambient temperature for a period of 30 days using fenugreek seeds and leaves powder.

Cakmak *et al.* (2015) observed no significant (P>0.05) differences in yeast and mould counts of snacks during the storage period of 3 months using chicken meat and chicken meat powder. Kasthuri *et al.* (2017) while evaluating the quality of chicken chips during storage at room temperature for a period of 30 days where no significant difference was found in yeast and mould between the storage intervals. Absence of yeast and mould in chicken chips prepared in the current study indicated that good processing procedures, storage techniques and keeping conditions were followed while preparing the product. Thus the product could be safely considered for consumption.

4.6.8. Sensory evaluation for organoleptic quality

4.6.8. i) Colour score

The mean (±SE) colour scores of chicken chips under different treatment groups incorporated with fenugreek leaves and/or seeds powder are given in Table 4.99 and their analysis of variance in Table 4.100. All the products prepared and stored at room

temperature were periodically analyzed by taste panelists. It was interesting to know that the samples could retain good colour scores ranging from 5.5 ± 0.07 to 6.0 ± 0.25 (hedonic scale signifying good to very good score).

The freshly prepared chicken chips exhibited good scores in all the treatment groups along with the Control group. However no significant (P>0.05) differences were observed between the groups or when compared with the Control group. Numerically the higher scores seen in Control group might be due to the slight lighter in colour in comparison to the colour of the treatment groups. The characteristic reduction of colour might be due to the appearance of green leafy colour contributing to the slight discolouration of the product.

However, no significant changes were noted in colour scores with increase in storage period in all the groups. Similarly, Hegazy (2011) found no significant differences among beef burger samples in terms of color scores with storing the product up to 3 months.

TABLE 4.99: MEAN (±SE) COLOUR SCORES IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage period (Days)	Control	Treatment A (With 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	5.8 ± 0.29	5.6 ± 0.22	5.8 ± 0.29	6.0 ± 0.25
10	5.7 ± 0.33	5.8 ± 0.24	5.7 ± 0.26	5.8 ± 0.24
20	6.0 ± 0.25	5.7 ± 0.21	5.5 ± 0.22	5.5 ± 0.17
30	6.0 ± 0.25	5.5 ± 0.30	5.3 ± 0.30	5.5 ± 0.26

TABLE 4.100: ANOVA FOR COLOUR SCORES OF CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	1.95	0.65	0.943548^{NS}
Days	3	1.15	0.383333	0.556452^{NS}
Treatment X days	9	3.3	0.366667	0.532258^{NS}
Error	144	99.2	0.688889	

4.6.8.ii) Texture score

The mean (±SE) texture scores of chicken chips under different treatment groups incorporated with fenugreek leaves and/or seeds powder are given in Table 4.101 and their analysis of variance in Table 4.102. All the freshly prepared products and products stored at room temperature were periodically analyzed by taste panelists at 10 days interval up to a period of 30 days. It was fascinating to know that the samples could retain good texture scores ranging from 5.0 ± 0.34 to 6.2 ± 0.32. The hedonic score card signifying the chicken chips as good to very good product. A significant increase in texture score was observed in Treatment C group as compared to Control, Treatment A and Treatment B.

The texture scores of the chicken chips was found to be significantly ($P<0.05$) lower in Treatment- C group (5.9 ± 0.17) compared with other treatment groups and Control. This might be due to the presence of a hydrocolloid which is a soluble fibre in fenugreek changing the textural property of the product (Wani and Kumar, 2016).

TABLE 4.101:MEAN (±SE) TEXTURE SCORES IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	$5.3^{A} \pm 0.35$	$5.2^{A} \pm 0.36$	$5.0^{A} \pm 0.34$	$5.9^{B} \pm 0.17$
10	$5.5^{A} \pm 0.26$	$5.4^{A} \pm 0.14$	$5.9^{B} \pm 0.27$	$6.0^{B} \pm 0.29$
20	$5.6^{A} \pm 0.26$	$5.3^{A} \pm 0.26$	$5.8^{B} \pm 0.35$	$6.6^{B} \pm 0.42$
30	$5.3^{A} \pm 0.15$	$5.3^{A} \pm 0.15$	$5.7^{B} \pm 0.30$	$6.2^{B} \pm 0.32$

Means bearing different superscripts within a row differ significantly ($P<0.05$)

Similar reports were also obtained from results of Hegazy (2011) with improvement in texture scores in beef burger using fenugreek seed flour with increasing levels (3,6,9,12%). Qureshi *et al.* (2018) found the texture scores to be in increasing with increase in fenugreek seed up to 1.5% in spent hen meat patties. However, in the present study, no change in texture scores observed in the chicken chips throughout the storage period up to 30 days.

TABLE 4.102:ANOVA FOR TEXTURE SCORES OF CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	13.725	4.575	5.130841*
Days	3	1.425	0.475	0.53271^{NS}
Treatment X Days	9	4.225	0.469444	0.52648^{NS}
Error	144	128.4	0.891667	

4.6.8.iii) Crispiness score

The scores for crispiness of the chicken chips products are presented in the Table 4.103 and their ANOVA in Table 4.104.

No significant (P>0.05) differences were observed among the groups, however the panelists rated 5.5 ± 0.16 to 5.8 ± 0.13 scores out of 7 hedonic scale in all the fenugreek treated groups, Treatment-C scored the highest score (5.8 ± 0.13). Moreover the scores of the crispiness property remain comparable throughout the storage period of 30 days under ambient temperature packed using LDPE materials.

TABLE 4.103: MEAN (±SE) CRISPINESS SCORES IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	5.6 ± 0.33	5.8 ± 0.20	5.5 ± 0.16	5.8 ± 0.13
10	5.9 ± 0.23	6.0± 0.33	5.7 ± 0.21	5.7 ± 0.36
20	5.7 ± 0.26	5.6 ± 0.16	5.8 ± 0.29	5.5 ± 0.30
30	5.5 ± 0.30	5.9 ± 0.27	5.4 ± 0.33	5.6 ± 0.27

The crispness intensity and overall hedonic texture of dry snack food products are also a function of water activity (Katz and Labuza, 1981). Similar result were obtained by Singh *et al*. (2013) where the crispiness scores remained non-significant and were not affected by different levels (60-70%) of spent hen meat in ready-to-eat chicken meat caruncles. In another study Singh *et al.* (2011) found non-significant change in crispiness scores in chicken snacks stored at aerobic (30 ± 2^0C) and vacuum packaging up to 30 days.

TABLE 4.104: ANOVA FOR CRISPINESS SCORES OF CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	1.125	0.375	0.509434^{NS}
Days	3	1.125	0.375	0.509434^{NS}
Treatment X Days	9	2.125	0.236111	0.320755^{NS}
Error	144	106	0.736111	

4.6.8.iv) Flavour score

The mean (±SE) flavour scores of chicken chips under different treatment groups incorporated with fenugreek leaves and/or seeds powder are given in Table 4.105 and their analysis of variance in Table 4.106. Addition of fenugreek seeds and leaves powder in preparation of chicken chips showed the flavor scores ranged from very poor score of 2.1 ± 0.76 up to a good score of 5.6± 0.34 under hedonic scale. Significantly (P<0.01) lower after-taste scores could be observed in Treatment B and C as 2.4± 0.26 and 2.3 ± 0.36, respectively on the day of preparation compared to Control and Treatment A.

TABLE 4.105: MEAN (±SE) FLAVOUR SCORES IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage period (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	5.6^{A}± 0.34	5.0^{A} ± 0.29	2.4^{B}± 0.26	2.3^{B} ± 0.36
10	5.4^{A} ± 0.26	4.9^{B}± 0.31	2.8^{C} ± 0.35	2.1^{C} ± 0.27
20	5.3^{A} ± 0.33	4.4^{B} ± 0.37	2.6^{C} ± 0.71	2.1^{C} ± 0.76
30	5.5^{A} ± 0.22	4.3^{B} ± 0.33	3.0^{C} ± 0.33	2.5^{C} ± 0.34

Means bearing different superscripts within a row differ significantly (P<0.01)

However no significant changes were observed among all the groups with increase in storage period up to 30 days under aerobic condition.

The reduction in flavor scores might be due to the increase in bitterness of the product with increase in fenugreek content of the chicken chips. The occurrence of bitterness of the chicken chips was mostly due to the presence of alkaloids mainly Trigonelline (Muraki *et al.* 2012). Comparable results were also found in the study of Qureshi *et al.* (2018) with reduction in flavor scores.

TABLE 4.106: ANOVA FOR FLAVOUR SCORES OF CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	278.7188	92.90625	94.41426**
Days	3	1.31875	0.439583	0.446718^{NS}
Treatment X Days	9	5.75625	0.639583	0.649965^{NS}
Error	144	141.7	0.984028	

4.6.8.v) After taste score

The scores and analyses of variance for after-taste have been given in Table 4.107 and analysis of variance in Table 4.108. The taste panelists rated from the product from 1.9 ±0.76 to 5.6 ±0.22 out of hedonic scale score of 7. The present study revealed that there no significant differences between the Control and the Treatment A group, incorporated with 0.25% fenugreek leaves powder. However significant change in after-taste was noted in the Treatment B and Treatment C as compared with Control and Treatment A.

The after-taste scores were found to have decreased significantly with increase in fenugreek content of the product in Treatment B and Treatment C. However no significant changes could be observed in the scores of after-taste of the chicken chips throughout the storage period. It has been noted that very low scores were obtained in Treatment group treated with the increased levels of fenugreek seed and/or leaves powder. The highest scores were rated to the Control group (5.3± 0.34 to 5.6 ± 0.22)**.**

Panelists gave a range of 'good' scores to the Treatment A and Control groups; whereas, a range of 'poor' scores to the Treatment B and C. This might be because of their perception of bitterness of the product due to the addition of fenugreek leaves or seeds (Kasthuri *et al*., 2018)

TABLE 4.107: MEAN (±SE) AFTER-TASTE SCORES IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage interval (Days)	Control	Treatment A (with 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	5.3^{A}± 0.34	5.2^{A} ± 0.29	3.6^{B} ± 0.26	2.3^{C} ± 0.36
10	5.3^{A} ± 0.26	5.0^{A} ± 0.31	3.3^{B} ± 0.35	2.1^{C} ± 0.27
20	5.3^{A} ± 0.33	5.0^{A} ± 0.37	3.2^{B} ± 0.71	1.9^{C} ± 0.76
30	5.6^{A} ± 0.22	5.1^{A} ± 0.33	3.3^{B} ± 0.33	2.3^{C} ± 0.34
Means bearing different superscripts within a row differ significantly (P<0.01)				

TABLE 4.108: ANOVA FOR AFTER-TASTE SCORES OF CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Treatment	3	275.625	91.875	103.8462**
Days	3	1.725	0.575	0.649922^{NS}
Treatment X Days	9	1.225	0.136111	0.153846^{NS}
Error	144	127.4	0.884722	

4.6.8.vi) Overall acceptability score

The panelists rated the overall acceptability of the freshly prepared chicken chips with good score (5.1 ± 0.17 to 5.8 ± 0.35) for the control group (Table 4.109). However, the scores decreased significantly (P<0.05) in the Treatment groups, A, B and C. The overall acceptability of the product gradually decreased with increase in fenugreek content which significantly (P<0.01) pronounced in Treatment-C containing both fenugreek-leaves and -seeds powder. All the treatment and the Control groups maintain comparable score throughout the storage period of 30 days under aerobic condition.

TABLE 4.109: MEAN (±SE) OVERALL ACCEPTIBILITY SCORES IN CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Storage interval (Days)	Control	Treatment A (With 0.25% FL)	Treatment B (With 0.25% FS)	Treatment C (0.25% FL+FS)
0	5.8^{A}± 0.35	4.9^{B} ± 0.45	3.5^{C} ± 0.52	2.2^{D} ± 0.35
10	5.1^{A} ± 0.17	4.5^{B} ± 0.37	3.5^{C} ± 0.34	2.3^{D} ± 0.21
20	5.5^{A}± 0.37	4.1^{B} ± 0.56	3.5^{C} ± 0.42	2.2^{D} ± 0.29
30	5.5^{A} ± 0.22	4.6^{B} ± 0.26	3.1^{C} ± 0.43	2.3^{D} ± 0.21

Means bearing different superscripts within a row differ significantly (P<0.01)

TABLE 4.110: ANOVA FOR OVERALL ACCEPTIBILITY SCORES OF CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Source of Variation	df	SS	MS	F
Days	3	1.925	0.641667	0.476289^{NS}
Treatment	3	233.725	77.90833	57.82887**
Treatment X Days	9	5.125	0.569444	0.42268^{NS}
Error	144	194	1.347222	

The decline in overall acceptability scores could be reflective of changes in scores of flavour, colour, texture and other sensory attributes. Considering the health benefits and liking to the necessity of such products for the health benefits of diabetic consumers, the Treatment B and Treatment C with the level of 0.25% fenugreek seed power and 0.25+0.25% fenugreek seed and leaves combination, respectively can be vouched.

4.6.9. Cost of production

The cost of production of chicken chips per kg using spent hen meat, incorporated with fenugreek seeds or leaves powder in the present study are calculated to be in the range from ₹ 248.30 to 248.75 (Table 4.111). This cost was as per the market prices of ingredients used in Guwahati city. The market scenario might be different in other places as the cost of spent hen meat varies. Thereby the cost of production of

chicken chips made using spent hen meat might change accordingly. Moreover, the method for packaging and preservation are simple and can be effectively stored at room temperature for a period of 30 days. It was also found that no microbiological and physiological changes could be detected in the product during the entire day of storage. From the present study, 0.25% fenugreek leaves powder incorporated chicken chips was found to be the most preferred treatment group with outstanding sensory scores and fulfilling all the physicochemical requirements. It also showed that the product, ready-to-eat chicken chips (30g) cost approximately ₹7.45. The commercially available chips in the Guwahati city of the same size are about ₹10.00-15.00. Thus, it gives a good scope for the commercialization of the product with a fair profit margin and could be developing a healthy version of chips for the consumers. Although the other treatment groups did not attained satisfying results in terms of sensory scores, yet these products can be safely used for human consumption. These chicken chips proved to have some amount of health benefits and can be consumed by diabetics and other heath compromised patients and can relish it gladly.

TABLE 4.111: COST OF PRODUCTION OF CHICKEN MEAT CHIPS UNDER DIFFERENT TREATMENT GROUPS

Sl No	Ingredients (Rs./kg)	Control	Treatment A	Treatment B	Treatment C
1	Cost of deboned meat @ ₹300.00	180.00	179.25	179.25	179.99
2	Cost of Bengal Gram flour @ ₹100.00	20.00	20.00	20.00	20.00
3	Cost of wheat flour @ ₹40.00	4.00	4.00	4.00	4.00
4	Cost of salt @ ₹ 20.00	0.50	0.50	0.50	0.50
5	Cost of spices @ ₹1000.00	20.00	20.00	20.00	20.00
6	Cost of condiments @ ₹ 200.00	10.00	10.00	10.00	10.00
7	Cost of baking powder @ ₹ 400.00	2.00	2.00	2.00	2.00
8	Cost of vegetable oil @ ₹ 200.00/litre	2.00	2.00	2.00	2.00
9	Processing cost @ ₹10.00	10.00	10.00	10.00	10.00
10	Fenugreek leaves @ ₹400.00	0.00	1.00	0.00	0.50
11	Fenugreek seeds @ ₹250.00	0.00	0.00	0.625	0.3125
	Total cost of ready-to-eat chips (₹./kg)	**248.50**	**248.75**	**248.38**	**248.30**
	Cost of production of 30g packet (ready-to cook chips)	**≈ ₹7.45**			

CHAPTER - V

Summary and Conclusion

DEVELOPMENT OF READY-TO-COOK CHICKEN CHIPS USING SPENT HEN MEAT INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

CHAPTER-V

SUMMARY AND CONCLUSIONS

The present study was conducted to develop ready-to-cook chicken chips utilizing spent hen meat incorporated with fenugreek seeds and/or leaves powder. For this study 20 (twenty) healthy spent hens were used for the preparation of chicken chips following standard procedures of slaughtering and processing.

Fenugreek (*Trigonella foenum graecum*) seeds and its fresh leaves were purchased from local market of Guwahati city. Both of them were washed separately under running tap water for cleaning. The seeds were dried at 55-60°C for 2 hours, while the leaves were dried at 40°C for two days in hot air oven. The leaves and the seeds were ground separately into fine powder in an electric grinder, sealed hermetically and stored in plastic containers in the food cabinet of refrigerator for further use.

The fenugreek leaves and the seeds were analyzed for proximate parameters. The fenugreek leaves contained 85.64 ± 0.72% moisture, 4.62 ± 0.14% protein, 0.94 ± 0.01% ether extract, 1.69 ± 0.13% crude fibre and 10.73 ± 0.12% total ash. While the fenugreek seeds contained 10.26 ± 0.15% moisture, 26.86 ± 0.10% crude protein, 10.72 ± 0.15% ether extract, 47.52 ± 0.39% crude fibre and 3.82 ± 0.07% total ash.

The qualitative phytochemical studies of fenugreek seeds and leaves were carried out. Both of them were found positive for major phytochemicals, such as steroids, phenols, tannins, flavanoids, alkaloids and saponins.

The antioxidant properties of the fenugreek seeds and leaves were carried out using ethanolic extract. For this, 250g of seed powder and leaves powder was weighed and mixed separately with 500 ml of ethanol (99.90%) in conical flasks. The flasks were then kept in a cupboard in dark for one week and filtered by using muslin cloth. The filtrate was allowed for evaporation by using hot water bath at a temperature of 63°C for approximately 4 hours. The DPPH radical scavenging activity of fenugreek seeds and leaves were measured using the methods of Soni and Sosa (2013).

The mean per cent value of inhibition of DPPH radical by ethanolic extract at 50 µg/ml concentration were observed to be 40.04 ± 0.13 and 54.77 ± 0.14% for fenugreek leaves and fenugreek seeds, respectively. The mean per cent inhibition of DPPH radical by ethanolic extract at 100 µg/ml concentration were observed to be 44.11 ± 0.09 and 60.96 ± 0.16% for fenugreek leaves and fenugreek seeds, respectively. The mean per cent inhibition of DPPH radical by ethanolic extract at 150 µg/ml concentration were observed to be 56.42 ± 0.12 and 66.46 ± 0.11% for fenugreek leaves and fenugreek seeds, respectively. The mean per cent inhibition of DPPH radical by ethanolic extract at 200 µg/ml concentration were observed to be 65.03 ± 0.14 and 75.38 ± 0.08% for fenugreek leaves and fenugreek seeds, respectively. The ethanolic plant extracts derived from both the seeds and leaves were found to exhibit impressive antioxidant activity.

The total phenolic content in ethanolic extract at 50 µg/ml concentration were observed to be 4.04 ± 0.13 and 14.98 ± 0.06 mg/GAE for fenugreek leaves and fenugreek seeds, respectively. The mean per cent total phenolic content by ethanolic extract at 100 µg/ml concentration were observed to be 4.71 ± 0.14 and 15.16 ± 0.04 mg GAE/g for fenugreek leaves and fenugreek seeds, respectively. The mean per cent total phenolic content by ethanolic extract at 150 µg/ml concentration were observed to be 4.99 ± 0.07 and 15.17 ± 0.03 mg GAE/g for fenugreek leaves and fenugreek seeds, respectively. The total phenolic content in ethanolic extract at 200 µg/ml concentration were observed to be 5.09 ± 0.09 and 15.20 ± 0.04 mg GAE/g for fenugreek leaves and fenugreek seeds, respectively. High phenolic content in both fenugreek leaves and sees supports to the logic of using them as potential antioxidative agents in chicken chips.

The mean (±SE) Ferric reducing activity by ethanolic extract at 100 µg/ml concentration were observed to be 0.25 ± 0.02 and 0.47 ± 0.06 for fenugreek leaves and fenugreek seeds, respectively. The reducing activity by ethanolic extract at 200 µg/ml concentration was observed to be 0.27 ± 0.02 and 0.53 ± 0.02 for fenugreek leaves and fenugreek seeds, respectively. The mean Ferric reducing activity by ethanolic extract at 300 µg/ml concentration were observed to be 0.35 ± 0.19 and 0.71 ± 0.04 for fenugreek leaves and fenugreek seeds, respectively. The reducing activity by ethanolic extract at 400 µg/ml concentration were observed to be 0.51 ± 0.05 and 0.89 ± 0.05% for

fenugreek leaves and fenugreek seeds, respectively. Elevated ferric reducing activity of both fenugreek leaves and seeds indicates their high powers as antioxidants.

The antibacterial activities of both extracts (fenugreek leaves and seeds) exhibited positive reaction against *Staphylococcus aureus* and *Klebsiella* spp. at different concentrations. The diameter of the zone of inhibition was recorded in millimeter using a transparent scale. The zone of inhibition against *Staphylococcus aureus* ranged from 11 to 19 mm for fenugreek leaves and from 10 to 15 mm for fenugreek seeds. Likewise, the zone of inhibition against *Klebsiella* spp. ranged from 12 to 15 mm for fenugreek leaves and 13 to 16 mm for fenugreek seeds. This study provides evidence that crude extracts of fenugreek seeds exhibited anti-bacterial effect against *E. coli* but no effect could be found with fenugreek leaves. In the present study, no zone of inhibition could be observed for fenugreek leaves as well as fenugreek seeds against *Salmonella* spp.

Following this, the research trials were continued in two Phases, i.e., I and II. Under Phase I chicken chips was prepared as per standard formulation incorporating fenugreek seeds and/or leaves @ 0.25, 0.50 or 1.00 % level. The chicken chips were preserved in sealed LDPE bags at ambient temperature (37 ± 2^0C) for a period of 30 days. The samples were evaluated for the physicochemical, proximate and sensory parameters at a regular interval of 10 days till 30^{th} day.

The moisture level in all the treatment groups for fenugreek leaves as well as seeds including that of control progressively increased as storage period extended till 30^{th} day. Under Control group, the values significantly increased from 0 day to 20^{th} day beyond which there was no significant change. Under Treatment-I (with 0.25% FL) the moisture level did not increase till 10^{th} day of storage. However, significant increase in moisture was recorded on 20^{th} and 30^{th} day of study. Similar trend was observed in the samples Treatment II (With 0.50% FL) and III (With 1.00% FL). However there was no significant difference among the four treatments including that of Control. The change of moisture level in the stored chicken chips using fenugreek seed powder followed the same trend as described for the product using fenugreek leaves.

The crude protein levels in chicken chips added with fenugreek leaves ranged from 22.33 ±0.22 to 22.44 ±0.08% values. The analysis revealed no significant (P>0.05) differences among the various treatment groups. The chicken chips incorporated with fenugreek seed powder found to have mean (±SE) crude protein ranged from 22.33 ±0.22 to 22.85±0.09% . The protein percentage in the treatment groups (IV, V and VI) showed to have increased with increase in fenugreek seeds level (0.25, 0.50 and 1.00%) as compared to the Control.

The chicken chips incorporated with fenugreek leaves powder and control group found to have ether extract ranged from 4.81±0.06 to 4.88±0.03% and for chicken chips incorporated with fenugreek seeds had ether extract ranged from 4.81±0.06 to 4.93 ± 0.02%. The statistical analysis revealed no significant (P>0.05) differences among the various treatment groups and with increasing storage period.

The chicken chips incorporated with fenugreek leaves powder and control group had mean (±SE) total ash ranged from 7.58±0.02 to 7.60±0.00%. For the chicken chips incorporated with fenugreek seeds powder and control group contained mean (±SE) total ash ranged from 7.58±0.02 to 7.72 ± 0.03%. The analysis revealed no significant (P>0.05) changes among the various treatment groups added with fenugreek leaves whereas significant differences (P<0.05) could be noted in chicken chips incorporated with fenugreek seeds with increase in the level of fenugreek seed powder (0.5 and 1.0%).

In the present study, all the treatment groups incorporated with fenugreek leaves along with the control group up to the duration of 20^{th} days of storage showed pH values ranging from 5.65 ± 0.01 to 5.68 ± 0.01. No significant changes could be found between the different treatment groups up to 20^{th} day of storage. Significant increase in pH could be seen in 30^{th} day of storage in all the treatment groups including that of Control (5.62± 0.02 to 5.65 ± 0.02). Similarly, all the treatment groups with fenugreek seeds along with the control group up to the duration of 20^{th} days of storage showed pH values ranging from 5.61±0.01 to 5.68 ± 0.04. No significant changes could be found between the different treatment groups and Control up to 20^{th} day of storage except Treatment VI with 1.0% fenugreek seeds.

In the present study, it was observed that the tyrosine values ranged from 4.15 ± 0.03 to 4.19 ± 0.02 for chicken chips incorporated with fenugreek leaves powder; whereas the values ranged from 4.15 ± 0.03 to 4.27 ± 0.02 for chicken chips incorporated with fenugreek seed powder. The impact of storage could not be noticed in the products made of, either leaves or seeds.

The water activity of the product was found to be in the range of 0.67 ± 0.02 to 0.73± 0.00 for chicken chips incorporated with fenugreek leaves powder. Whereas chicken chips incorporated with fenugreek seed powder revealed 0.67 ±0.01 to 0.72 ±0.02. The water activity remained unchanged till the 20^{th} day of storage, however increased significantly ($P<0.05$) on 30^{th} day. The water activity in the chips prepared using fenugreek leaves powder or fenugreek seeds powder behaved in a similar fashion as the storage period extended. Further, the level of fenugreek leaves or fenugreek seeds did not have any impact on the water activity of the products.

The cooking yield of 90.97 ± 0.76% to 95.00 ± 1.77% range was recorded in the chicken chips incorporated with fenugreek leaves; whereas, 91.16 ±0.78% to 93.59 ± 0.18% range was noted in the chicken chips incorporated with fenugreek seeds powder.

These values remained statistically comparable. The analysis revealed no significant ($P>0.05$) differences among the various treatment group and with storage time.

The freshly prepared chicken chips with addition of fenugreek leaves and seeds on day 1 exhibited 'good' colour scores (5.3 to 5.6 out of 7 hedonic scale) for all the chips under different treatment groups incorporated with fenugreek leaves. The freshly prepared chicken chips added with fenugreek seed powder at the different levels of 0.25, 0.50 and 1.0 % exhibited 'good' scores in all the treatment groups along with the control group ranging from 5.50 ± 0.22 to 5.70 ± 0.33 out of 7 score of the hedonic score card.

The texture scores for the fenugreek leaves treated chicken chips found to have no significant deviation from the control group. The texture scores obtained by the product chicken chips incorporated with fenugreek leaves and/or seeds powder showed a 'good' score for texture. The texture recorded to have score from 5.40 ± 0.26 to 5.60 ±

0.31 for chicken chips incorporated with fenugreek leaves powder. Whereas scores from 4.80 ± 0.32 to 5.10 ± 0.35 for chicken chips incorporated with fenugreek seeds powder were recorded.

In the present study, 'good' scores for crispiness was obtained in all the treatment groups including the control group (5.8 ± 0.25 to 6.2 ± 0.36) out of 7 score in hedonic scale for chicken chips product added with fenugreek leaves. Similarly texture scores for chicken chips incorporated with fenugreek seed powder were found in the range of 5.8 ± 0.25 to 6.2 ± 0.36.

However the crispiness scores for the fenugreek seeds and leaves treated chicken chips found to have no significant deviation from the control group.

From the data obtained after sensory evaluation of the chicken chips product treated with fenugreek seeds and leaves powder showed low flavour scores in the Treatment II & III and Treatment V & VI groups throughout the storage period up to 30 days.

From the data, it can be noted that the chicken chips without addition of fenugreek and Treatment I with 0.25% fenugreek seeds and leaves showed no changes in after-taste score. However, increasing the level of fenugreek leaves powder reduced the score for after-taste from 5.00 ± 0.37 to 2.80 ± 0.36 out of 7 scale of hedonic score card. With the increase in fenugreek seed content from 0.25 to 1.00% level in chicken chips the scores for after-taste have gradually decreased from 6.10 ± 0.18 to 2.10 ± 0.31.

The overall acceptability scores for chicken chips using spent hen meat incorporated with fenugreek seed and leaves powder are found to be within the range of 2.00 ± 0.33 to 5.50 ±0.34 which fall from very 'poor' to 'good' scores for chicken chips incorporated with fenugreek leaves. The overall acceptability scores for chicken chips incorporated with fenugreek seed ranged from 5.90 ± 0.38 to 2.10 ± 0.35.

Under Phase I trial, based on the statistical analysis obtained, two best groups mentioned below were selected for further studies. All the physicochemical values for the treatment groups were found to be under desirable ranges.

The tabulated data of the mean moisture content of the chicken chips sing spent hen meat are within the range of 4.36 ± 0.03 to 4.54 ± 0.06%. The analysis of variance revealed a significant ($P<0.05$) increase in moisture per cent with increase in storage period. It was interesting to note that there was no significant change in all the treatment groups of chicken chips including that of Control from 0 to 20^{th} day of storage irrespective of the levels of herbs used. However significant increase in the moisture level was found on 30^{th} days of storage as compared to the 0^{th}, 10^{th} and 20^{th} day values. However, no such change was observed among different treatment groups.

The crude protein values ranged from 22.36 ± 0.02 to 23.03 ± 0.06% among all treatment groups.. The analysis showed a significant ($P<0.05$) increase in crude protein per cent in Treatment-B and Treatment-C when compared with Control and Treatment A group. However, the values remained incomparable during the storage periods.

The ether extract content in chicken chips averaged between 4.65 to 4.73%. The mean value of the groups Treatment A, B and C revealed non-significantly ($P>0.05$) changes when compared with the Control group.

The total ash content of this unique product ranged from 7.41 ± 0.02 to 7.47 ± 0.00% after incorporation of fenugreek seeds and/or leaves powder in the chicken chip formulation. It was found that there was no significant difference in the total ash content in the chicken chips after incorporation of fenugreek seeds and/or leaves powder among all the treatment groups and the Control. Moreover, there were also no significant changes in the total ash content with progression of storage period up to 30 days.

The pH of all the treatment groups of product chicken chips incorporated with fenugreek leaves and/or seeds powder including that of Control ranged from 5.58 ± 0.05 to 5.69 ± 0.01%. The pH values of all the samples remained comparable with non-significant ($P>0.05$) differences up to 20^{th} day of storage. However on 30^{th} day, significant change in pH values could be recorded in all the treatment groups including that of Control. Storage days showed significant ($P<0.05$) effect on pH of the dried products. Treatment-C had significantly lower ($P<0.05$) pH on 30^{th} day when Compared with 0^{th} to 20^{th} day of storage.

It was observed that the tyrosine values ranged from 4.95 ± 0.06 to 5.17 ± 0.02%. The analysis of variance revealed no significant difference (P>0.05) in tyrosine value among the treatments and among the storage periods. There was no significant difference between the treatment groups and Control group throughout the storage period and the values remained far below permissible limit for all the products.

The water activity of the chicken chips recorded ranged from 0.683 ± 0.00 to 0.716 ± 0.00. The analysis of variance revealed significant difference (P<0.05) in water activity values on 30^{th} day of storage compared to the a_w on the day of preparation and 10^{th} and 20^{th} day of storage. The water activity remained safe from 0^{th} to 20^{th} day of storage whereas increased significantly on 30^{th} day. Highest a_w was recorded (0.716) in the Treatment B group, however within permissible limit. No significant changes in water activity parameter were found in all the treatment groups and compared to Control when stored up to 20^{th} day. There were also no significant (P>0.05) changes in the values in the treatment groups as compared to the Control group.

The cooking yield ranged from 93.86 ± 0.33 to 94.17 ± 0.33% in all the treatment groups including the Control group. There have been no significant (P>0.05) differences in the cooking yield per cent of the chicken chips prepared from spent hen meat among the treatment groups and Control. Even there was no significant (P>0.05) change in cooking yield of the treatment groups with increase in storage period.

The TBA values was recorded to be 0.34 ± 0.02 for control and 0.32 ± 0.02, 0.28 ± 0.01 and 0.30 ± 0.01 for Treatment-A, Treatment-B and Treatment-C, respectively. It was interesting to note that the TBA values decreased significantly (P<0.05) on the 10^{th} day of storage and remained static thereafter. The herbal compounds present in the fenugreek leaves/or seeds must have contributed to bring this change. However, available literature is silent to elucidate this phenomenon.

The mean cholesterol content of ready-to-cook chicken chips using spent hen with addition of fenugreek seeds and fenugreek leaves are found to be as 30.55 ± 0 .14, 30.45 ±0.21, 30.39 ± 0.16 and 31.44 ± 0.14% for Control, T-A, T-B and T-C,

respectively. The ready-to-cook chicken meat chips made from spent hen recorded no significant (P>0.05) cholesterol values when compared to the Control group.

The L* values of the products ranged from 54.68 ± 1.45 to 61.99 ± 1.34 and showed decrease in values with the addition of fenugreek seed or leaves powder. The results revealed significantly higher values for lightness (P<0.05) in the control group than the other treatment groups.

There was no significant difference (P>0.05) in the Redness/Greenness (a*) value between control and treatments groups with addition of fenugreek seeds or leaves powder as well as among the treatments. The redness (a*) value of the chicken chips with the addition of fenugreek seeds found to be in the range from 8.65 ± 0.73 to 7.45 ±0.21 and values remained comparable.

In the current experiment with chicken meat chips, the values for yellowness ranged from 25.68 ± 1.17 to 27.54 ± 1.71 and showed no significant difference (P>0.05) among the treatments and Control groups.

In the present work, the mean DPPH radical scavenging activity were 17.03 ± 0.24, 20.41 ± 0.73, 20.89 ± 0.24 and 20.86 ± 0.31 for control, Treatment -A, Treatment -B and Treatment -C groups, respectively. There was an increase (P<0.05) in DPPH radical scavenging activity values with increase addition of fenugreek seeds or leaves powder in all the treatment groups as compared to the Control group. Moreover, a significant increase in DPPH activity noted with increase in concentration.

The antioxidant activity of chicken chips prepared using spent hen meat incorporated with fenugreek seeds and/or leaves at a concentration of 50µg/ml was found to be 1.840 ± 0.13 in control, 1.912 ± 0.14 in Treatment- A, 1.929 ± 0.16 in Treatment- B and 1.954 ± 0.23 in Treatment- C. At the concentration of 100µg/ml, the total phenolic content was found to be 1.887± 0.09 in control, 1.924 ± 0.16 in Treatment- A, 1.897 ± 0.11 in Treatment- B and 1.979 ± 0.19 in Treatment- C. At the concentration of 150µg/ml, the total phenolic content was found to be 1.890± 0.12 in control, 1.902 ± 0.11 in Treatment- A, 1.930 ± 0.17 in Treatment- B and 1.947 ± 0.16 in Treatment- C. At the concentration of 200µg/ml, the total phenolic content was found to be 1.897 ± 0.14 in

control, 1.924 ± 0.08 in Treatment- A, 1.944 ± 0.21 in Treatment- B and 1.971 ± 0.10 in Treatment- C. The mean antioxidant activity of chicken chips in terms of total phenolic content are recorded as 1.890 ± 0.27 in control, 1.916 ± 0.13 in Treatment- A, 1.925 ± 0.34 in Treatment- B and 1.963 ± 0.13 in Treatment- C.

There was no evidence of viable microbial growth in the chicken chips up to 20th day of storage under aerobic packaging. The data analysis described that there was an impact on the TPC values with storage period and packaging condition which was observed on 30th day of storage. However, there was an apparent growth of microorganisms on 30th day in all the treatment groups along with the control group though within safe limit. The TPC values obtained was significantly high in control when compared with Treatment B and Treatment-C. Whereas, no significant ($P>0.05$) difference was observed among the treatment groups.

The incidence of Salmonella was not detected in chicken chips incorporated with fenugreek seeds and/or leaves powder throughout the storage period of 30 days under aerobic condition.

The chicken chips incorporated with fenugreeks seed or leaves powder were subjected for Coliform bacterial count on day zero and subsequently at 10th, 20th and 30th day of storage under aerobic condition packing. The presence of Coliform bacteria was not detected in chicken chips incorporated with fenugreek seeds and/or leaves powder throughout the storage study of 30 days under aerobic condition.

The chicken chips incorporated with fenugreeks seed or leaves powder were subjected for Staphylococcal bacterial count on 0th, 10th, 20th and 30th day of storage under aerobic condition packing. No evidence of Staphylococcal counts was detected during storage period of 30 days in all the treatment groups including control group.

From the present study, it was fascinating to know that, there was no detection of yeast and mould in the chicken chips product stored at ambient temperature for a period of 30 days using fenugreek seeds and leaves powder.

The samples could retain 'good' colour scores ranging from 5.50 ± 0.07 to 6.00 ± 0.25 (hedonic scale signifying 'good' to 'very good' score). The freshly prepared chicken chips exhibited 'good' scores in all the treatment groups along with the Control group. However no significant ($P>0.05$) differences were observed among the groups or when compared with the Control group.

The samples could retain 'good' texture scores ranging from 5.00 ± 0.34 to 6.20 ± 0.32. The hedonic score card signifying the chicken chips as 'good' to 'very good' product. A significant increase ($P<0.05$) in texture score was observed in Treatment C group as compared to Control, Treatment A and Treatment B. Though the changes were noted in the groups, Treatment B and C the panelists judged the products to be 'good'.

All the crispiness scores of the chicken chips remained comparable, among the treatment groups, regardless of storage periods. The panelists rated 5.50 ± 0.16 to 5.80 ± 0.13 scores out of 7 hedonic scale indicating to be 'good'.

Addition of fenugreek leaves and/or seeds powder in preparation of chicken chips showed the flavor scores ranged from very poor (2.10 ± 0.76) to a good score (5.60± 0.34) under 7 point hedonic scale. The reduction in flavor scores might be due to the increase in bitterness of the product with increase in fenugreek content of the chicken chips.

The product under all the treatment groups secured the after-taste scores from 1.9 ± 0.76 to 5.6 ± 0.22 out of 7 hedonic scale. There no significant difference between the Control and the Treatment-A group incorporated with 0.25% fenugreek leaves powder. Whereas, significant changes in after-taste scores was noted in the Treatment-B and Treatment-C as compared with the Control and Treatment-A group.

The after-taste scores were found to have decreased significantly with increase in fenugreek content of the product in Treatment B and Treatment C. However no significant changes could be observed in the scores of the chicken chips throughout the storage period.

The overall acceptability of the freshly prepared chicken chips received 'good' score (5.1 ± 0.17 to 5.8 ± 0.35) for the control group. All the treatment and the Control groups maintained comparable score throughout the storage period of 30 days under aerobic condition. The overall acceptability of the product gradually decreased with increase in fenugreek content which significantly pronounced in Treatment-C containing both fenugreek-leaves and -seeds powder.

The cost of production of chicken chips per kg using spent hen meat incorporated with fenugreek seeds or leaves powder in the present study are recorded as approximately Rs. 249.00. It showed that the product, chicken chips (30g) cost approximately Rs. 7.45.

CONCLUSION

From the results and discussion of the present study, the following conclusions may be drawn.

The chicken chips prepared with the incorporation of spent hen and fenugreek leaves or seeds have revealed good antioxidant profile without any noticeable changes in any other physic-chemical parameters.

- The chicken chips prepared contained high protein per cent ranging from 22.36 ± 0.02 to 23.03 ± 0.06. This makes the product promising since the protein content in the chips available in local market is only 7-10%.

- Fenugreek leaves at 0.25% level can be effectively used in chicken chip preparation using spent hen meat with 'good' acceptability by sensory panelists up to 30 days of storage at ambient temperature. The product remained within the safe limit from microbiological safety standpoint.

- The cost of production for the preparation of ready-to-eat chicken chips incorporated with fenugreek leaves and/or seed powder are found to be Rs. 249.00 per kg or Rs. 7.445 per 30g at Guwahati market. Whereas the cost of ready-to-eat commercially available non-meat chips are Rs.10.00-15.00 per 30g. Thus, it created a good scope

for the commercialization of the product with a fair profit margin and could also be a healthy version of chips for the consumers.

- Inclusion of high level of fenugreek leaves and seeds powder (up to 1.00%) have decreased the palatability of the product. But can be recommended to consumers as a functional food with good medicinal values. Moreover, all the physio-chemical, antioxidant and antimicrobial parameters for the chicken chips with high levels of fenugreek leaves and seed powder fall within the safe ranges.

- The study can be further recommended to fortify the chicken chips using fenugreek leaves and/or seed extracts. Modified Atmospheric Packaging (MAP) may further enhance the shelf life and extend the horizon of marketing of the product.

Bibliography

DEVELOPMENT OF READY-TO-COOK CHICKEN CHIPS USING SPENT HEN MEAT INCORPORATED WITH FENUGREEK SEEDS AND/OR LEAVES POWDER

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